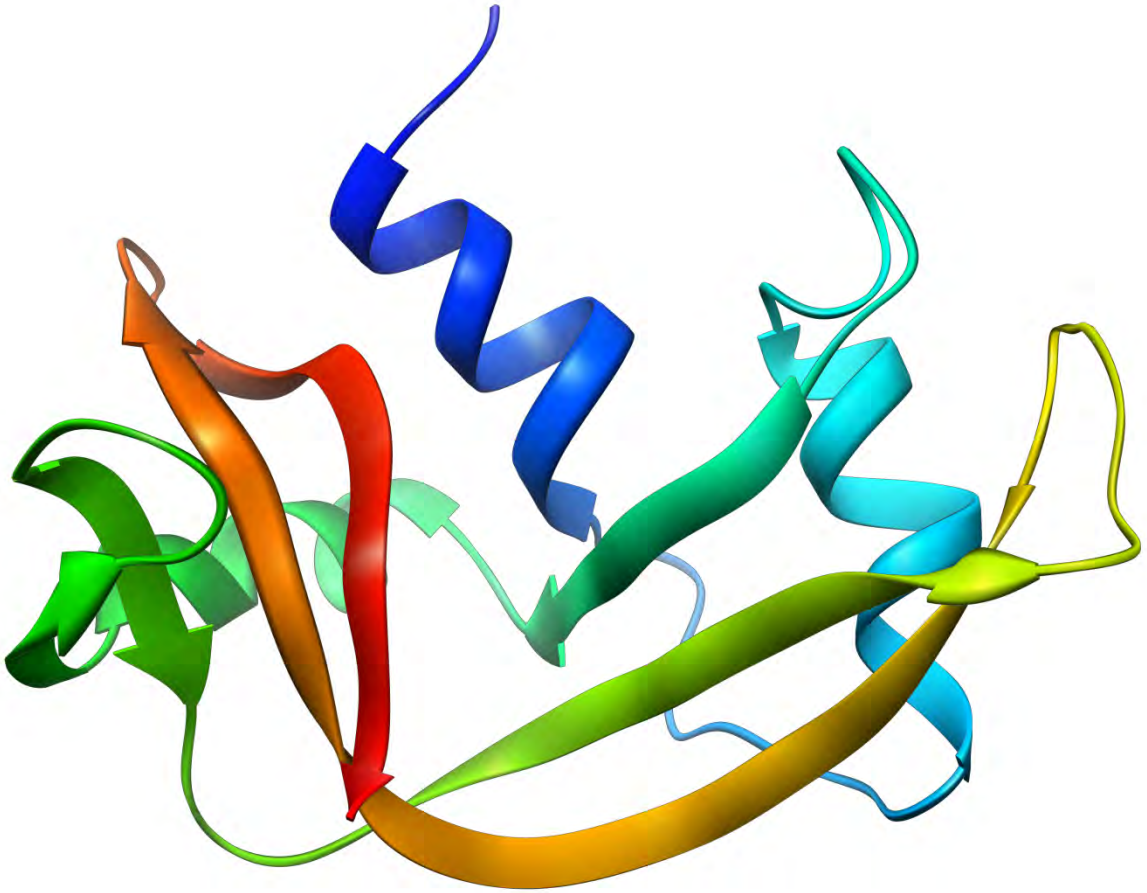
Protein Structure, Function and Disease

Non-covalent interactions, protein folding and role of water

(Partially adopted from Prof. John Baenziger's former lectures)

Jyh-Yeuan (Eric) Lee, Assistant Professor, BMI

Question: How does a protein fold from a newly synthesized linear polypeptide chain into a complex 3-dimensional structure?



Ribbon diagram of ribonuclease, a small globular protein that cleaves RNA. Christian Anfinsen used ribonuclease as a model in his pioneering studies of protein folding, which led to the Nobel Prize in Chemistry in 1972.

Christian Anfinsen's RNAse experiment

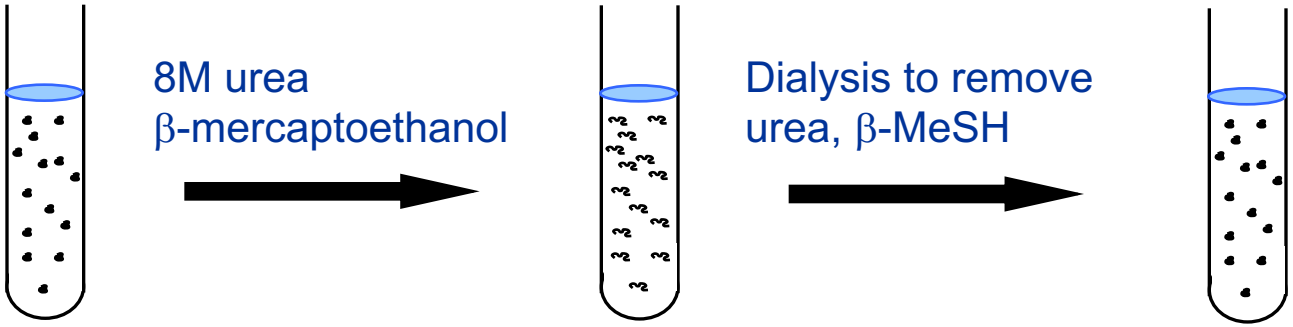
1972 Nobel Laureate Chemistry

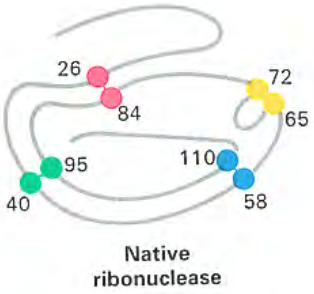
Christian B. Anfinsen

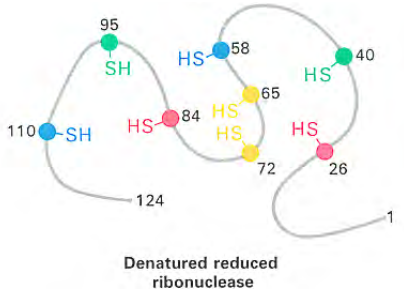
Nobel prize for showing that…

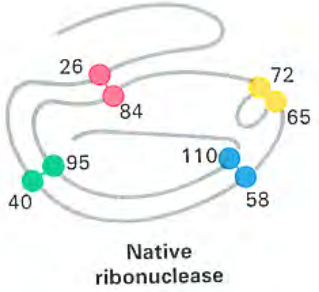
…the linear sequence of amino acids in the enzyme ribonuclease determines the biologically active conformation of this enzyme. This finding has profound implications for our understanding of the way in which active enzyme molecules are formed in living cells

Christian Anfinsen's RNAse experiment









Native Ribonuclease

BIOLOGICALLY ACTIVE

Denatured Ribonuclease

BIOLOGICALLY INACTIVE

Native Ribonuclease

BIOLOGICALLY ACTIVE

Anfinsen's work on protein folding showed that:

1) the amino acid sequence of a protein dictates the 3D fold

2) folding is a thermodynamic process – i.e. the folded state is the lowest energy conformation

3) the lowest energy conformation is the one in which the energy of the ***whole system*** **(not just the protein)** is the lowest

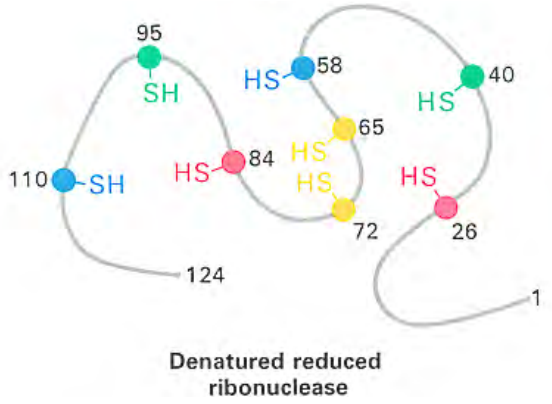
These three conclusions were summed up in what Anfinsen referred to as the Thermodynamic Hypothesis

The thermodynamic hypothesis:

"the three-dimensional structure of a native protein in its normal physiological milieu is the one in which the Gibbs free energy of the *whole system* is lowest; that is, that the native conformation is determined by the *totality of interatomic interactions* and hence by the amino acid sequence, in a given environment."

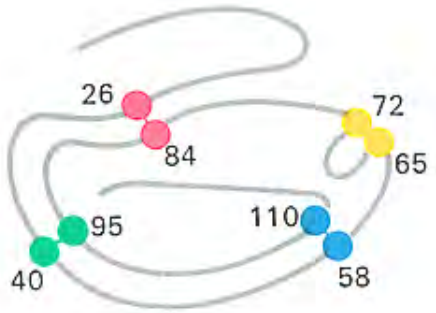
Excerpt from the Nobel Lecture "Studies on the principles that govern the folding of protein chains" given by Christian B. Anfinsen on December 11, 1972 : http://www.nobel.se/chemistry/laureates/1972/anfinsen-lecture.pdf

The thermodynamic hypothesis...

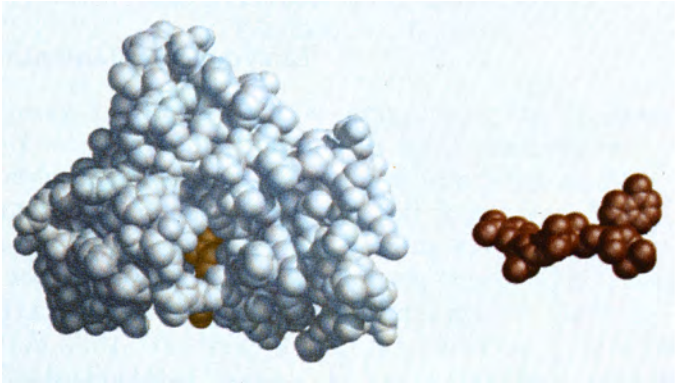


Denatured reduced ribonuclease

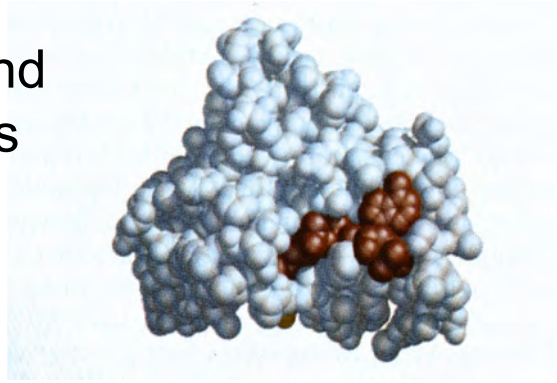
Protein folding



Native ribonuclease

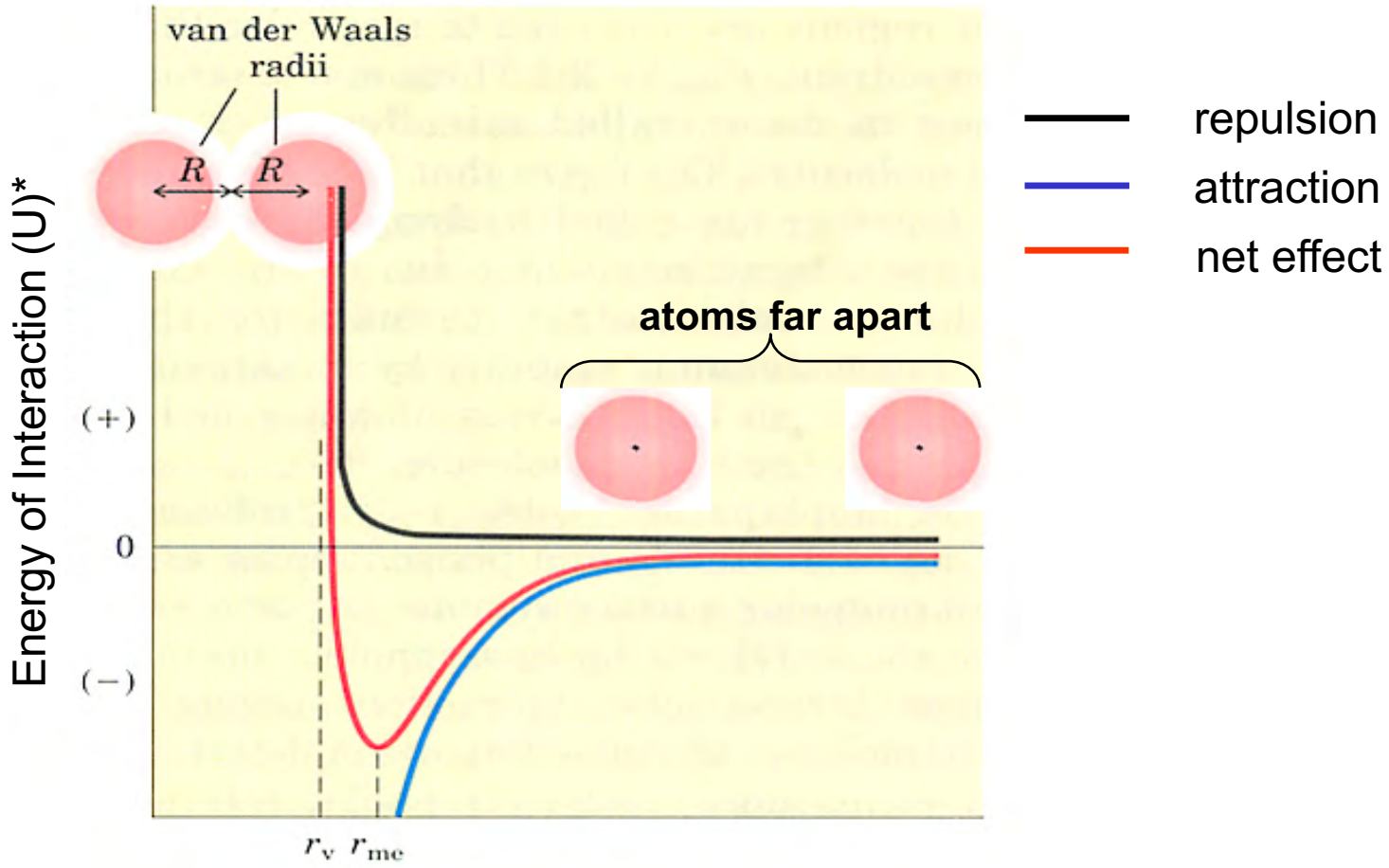


Protein-ligand Interactions



… governs all biological interactions including protein folding and protein-ligand interactions, etc. *But what does Anfinsen mean when he talks about the "whole system", the "totality of inter-atomic interactions"?*

Interactions between two atoms in a vacuum



*U is < 0 for an attractive interaction

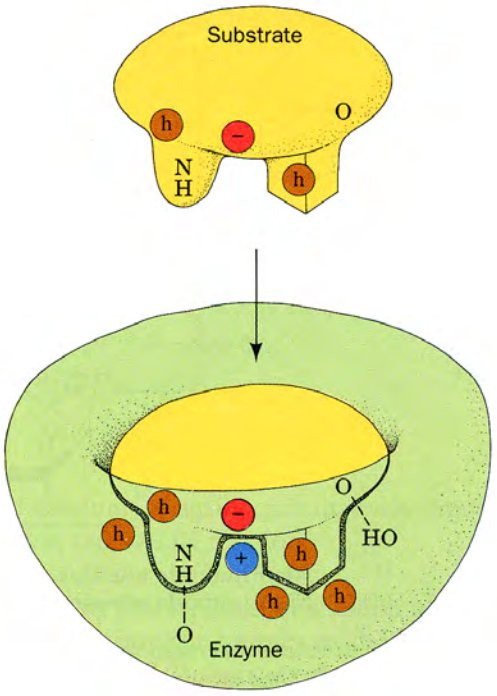
Interactions between two atoms in a vacuum

In this simple diatomic system in a vacuum, intermolecular interactions are governed by a BALANCE between repulsive (mainly steric, *black line*) and attractive (mainly electrostatic, *blue line*) interactions *(i.e. the totality of interactions")*. In this case the "whole system" refers to this balance between attractive and repulsive forces (*red line*).

Biological interactions are also a balance between repulsive and attractive forces, but the nature of these interactions is more complex:

- biological molecules are large and complex in terms of their structures (so some parts attract while others repel)
- biological molecules are dynamic
- *biological interactions typically occur in aqueous solution. When looking at the energy of the "whole system" we must consider the aqueous solvent!*

Molecular interactions are governed by:

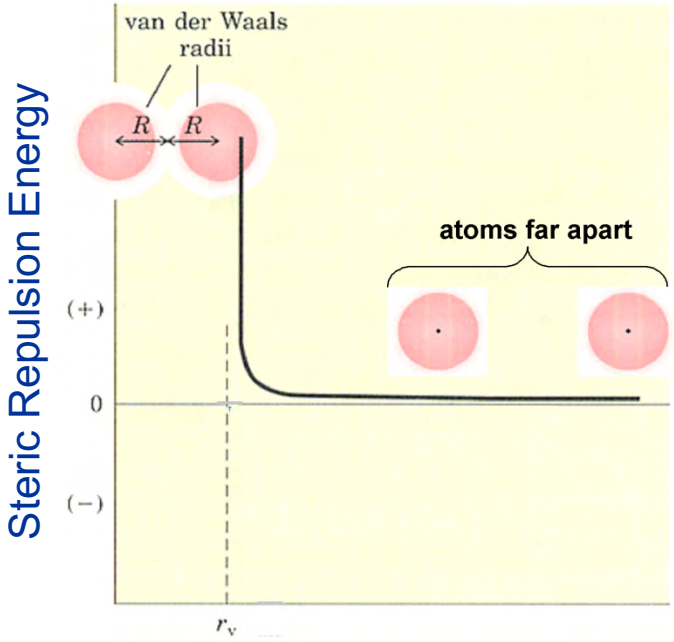


A schematic diagram of the forces that govern protein-ligand interactions.

- ***steric repulsion***
- electrostatic (salt bridge, charge-charge repulsion, van der Waals)
- hydrogen bond
- hydrophobic (h)

To understand protein function, we must understand the forces that drive inter-molecular interactions, but we must do so in the context of the whole system – which usually means an aqueous environment. Today, we focus on the forces that drive intermolecular interactions.

Steric repulsion



Distance between centers of atoms

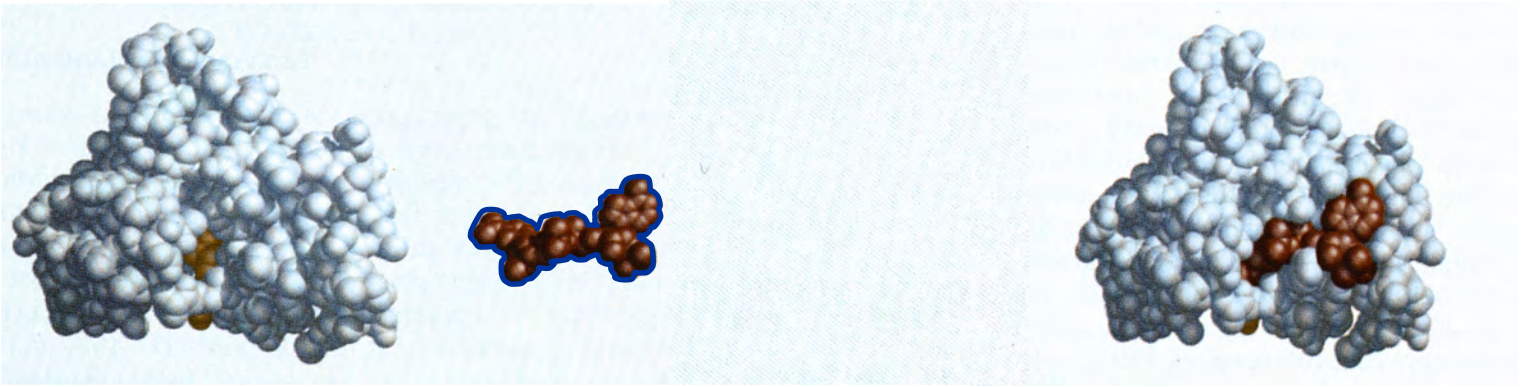
—— **repulsion**

$$U = \frac{B_{ij}}{r_{ij}^{12}}$$

r_{ij} is the distance between the centers of atoms i and j

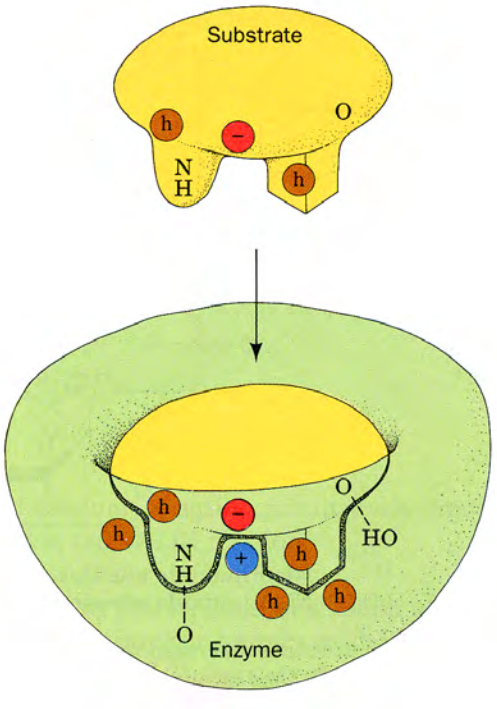
Steric repulsion occurs when two atoms come so close that their electronic orbitals begin to overlap. The resulting repulsion energy is so strong that we can consider atoms as impenetrable spheres at their "van der Waals radii". When the distance between two atoms approach their van der Waals radius, U quickly becomes infinite (*i.e. there is an inverse* r_{ij}^{12} *distance dependence*)!

Steric repulsion



Steric interactions play a major role in determining inter and intra-molecular interactions. For example, proteins have evolved binding pockets that have shapes that are complimentary to the shapes of specific ligands. *(Note that electrostatic interactions can also be repulsive as discussed below)*

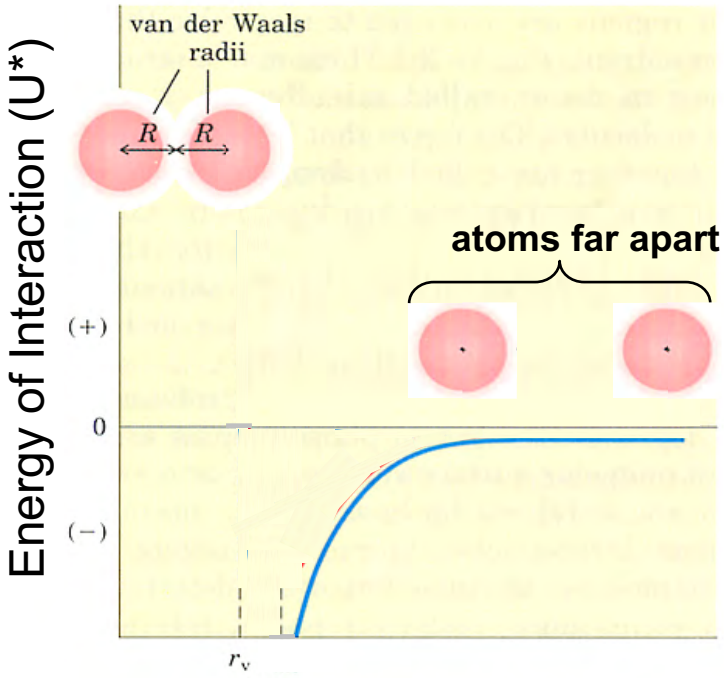
Molecular interactions are governed by:



A schematic diagram of the forces that govern protein-ligand interactions.

- steric repulsion
- ***electrostatic (salt bridge, charge-charge repulsion, van der Waals)***
- hydrogen bond
- hydrophobic (h)

Electrostatic interactions



attraction

$U = ??$

In biology, attractive interactions are typically electrostatic in nature. *Electrostatic interactions are non-covalent interactions between charged or partially charged molecules.* There are many types of electrostatic interactions.

Ionic interactions:

Ionic interactions are strong (40 to 400 kJmol^{-1}) electrostatic interactions between two charged molecules:

Aspartate *Lysine*

——CH_2-COO^- ··· ^+H_3N-CH_3——

Ionic interactions are governed by Coulomb's law, which states that:

1) opposite charges (*salt bridge)* attract and like charges repel
2) energy of interaction (U) between 2 point charges (q) depends on the distance between the charges according to:

Ionic interactions:

$$U = \frac{kq_1q_2}{r}$$

k = proportionality constant (9.0×10^9 JmC^{-2})
q = electronic charge ($\pm 1.6 \times 10^{-19}$ C)
r = distance between charges in meters (m)
(1 nm = 10Å)

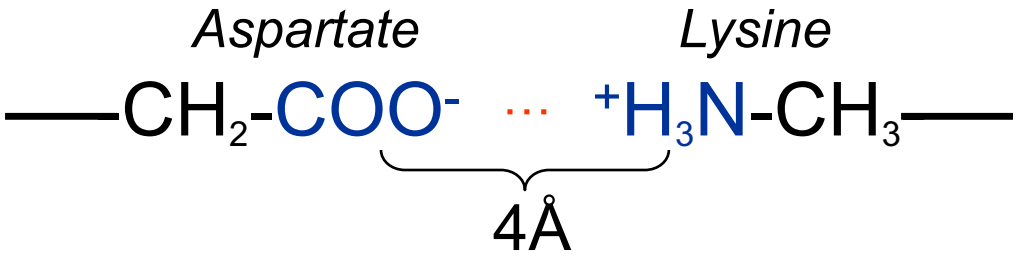
(assumes point charge, no motion, vacuum)

Note that the distance dependence of U is weak. Ionic interactions thus can be over large distances:

Ionic: $U \alpha 1/r$

Steric: $U \alpha 1/r^{12}$

Energy calculations



Calculate the energy of interaction between an aspartate and lysine in a vacuum at a distance of 4 Å assuming point charges and no motion:

$$U = \frac{kq_1q_2}{r}$$

k = 9.0 x 10^9 JmC^{-2}
q = ±1.6 x 10^{-19} C

$$U = \frac{(9.0x10^9\,JmC^{-2})(1.6x10^{-19}\,C)(-1.6x10^{-19}\,C)}{(4x10^{-10}\,m)}$$

$$U = (-5.76x10^{-19}\,J)(6.02x10^{23}\,mol^{-1})$$

$$\boxed{U = -347kJmol^{-1}}$$

van der Waals interactions:

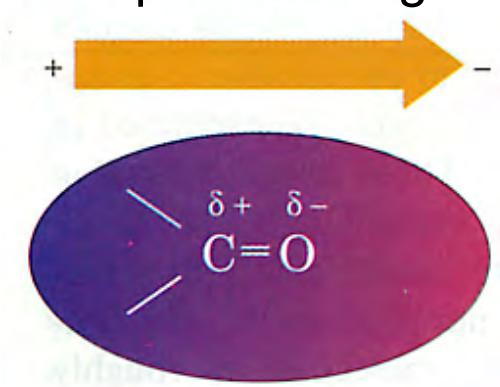
van der Waals interactions are weak noncovalent interactions between electrically neutral molecules (that may exhibit partial charges). They are electrostatic in nature:

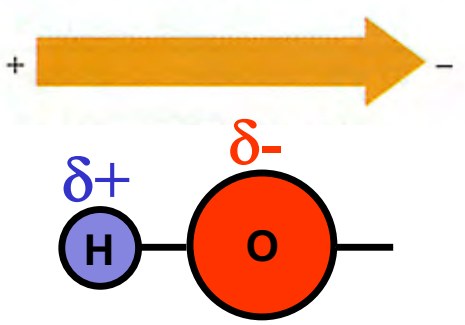
- dipole-dipole
- dipole-induced dipole
- induced dipole-induced dipole (London dispersion)
- *(ion-dipole and ion-induced dipole) – not van der Waals, but similar*

The interaction energies range from -0.3 to -4 $kJmol^{-1}$ and are roughly proportional to the inverse of the distance to the third power for fixed molecules ($U \alpha 1/r^3$), and to the sixth power for molecules in motion ($U \alpha 1/r^6$).

Dipole-dipole interactions

Electrically neutral molecules posses a dipole moment if there is a separation of partial charge





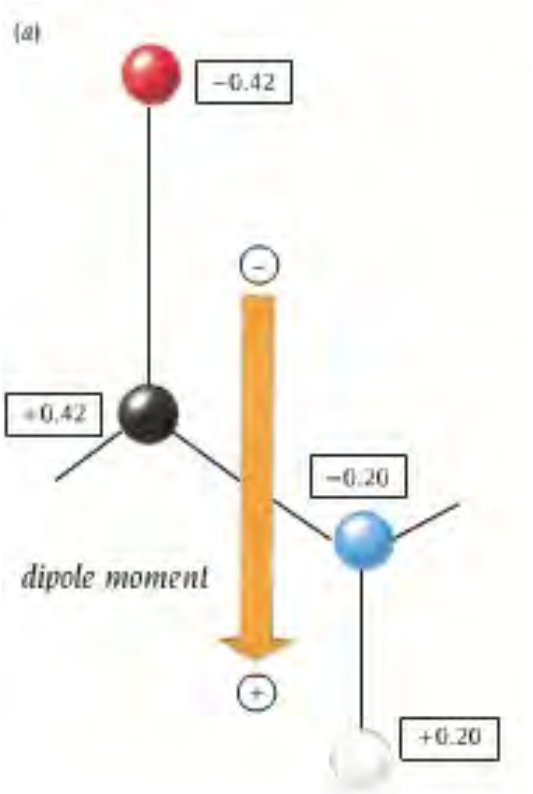
The dipole moment is a vector quantity (it has a direction) defined as the product between the partial charge and the distance between the partial charges

$$\vec{u} = q\vec{r}$$

The unit for dipole moment is Coulomb·meter, or Debye (D). The larger the value, the stronger the moment and thus the more polar the molecule

$$1\ D = 3.33564 \times 10^{-30}\ C \cdot m$$

Peptide bond possess a dipole moment



Both the C=O and N-H groups of the peptide bond exhibit dipoles. What is the dipole moment of the C=O group?

$$u = qr$$

The distance r between the carbon and oxygen is 1.24 Å. The charge is 0.42 of an electron charge: 0.42 x 1.6×10^{-19} C = 6.72×10^{-20}C.

$$u_{C=O} = 6.72x10^{-20}\,C\;x\,1.24x10^{-10}\,m$$

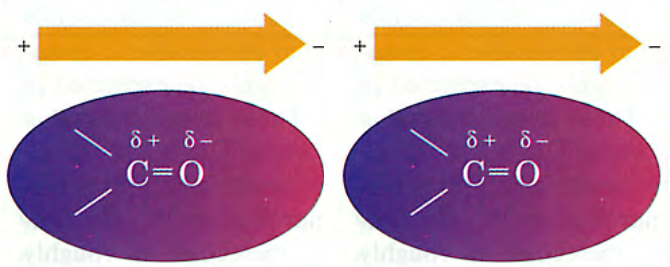
$$u_{C=O} = 8.33x10^{-30}\,C{\cdot}m \,/\, 3.33564x10^{-30}\,C{\cdot}m/D$$

$$u_{C=O} = 8.33x10^{-30}\,C{\cdot}m \,/\, 3.33564x10^{-30}\,C{\cdot}m/D$$

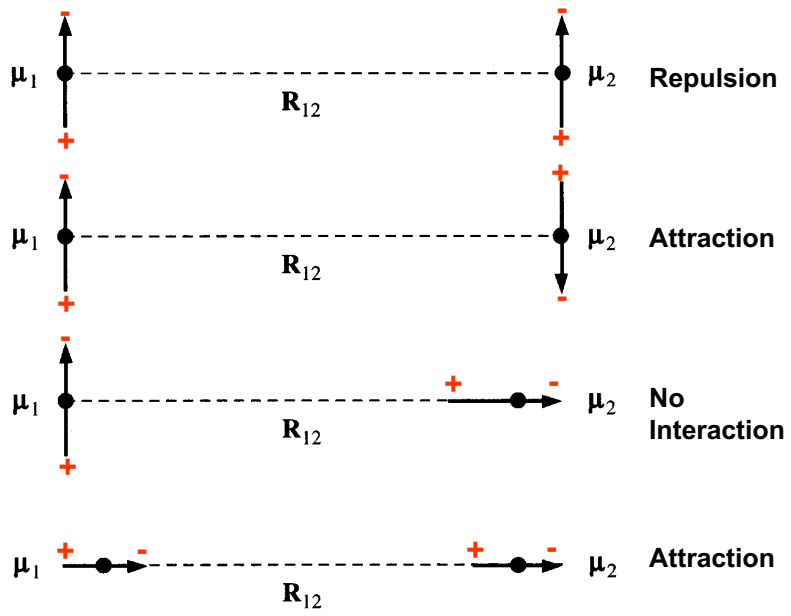
$$\boxed{u_{C=O} = 2.5Debye}$$

Dipole-dipole interactions

The strength of dipole-dipole interactions depends on the relative orientation of the two dipoles. They can be either attractive or repulsive:

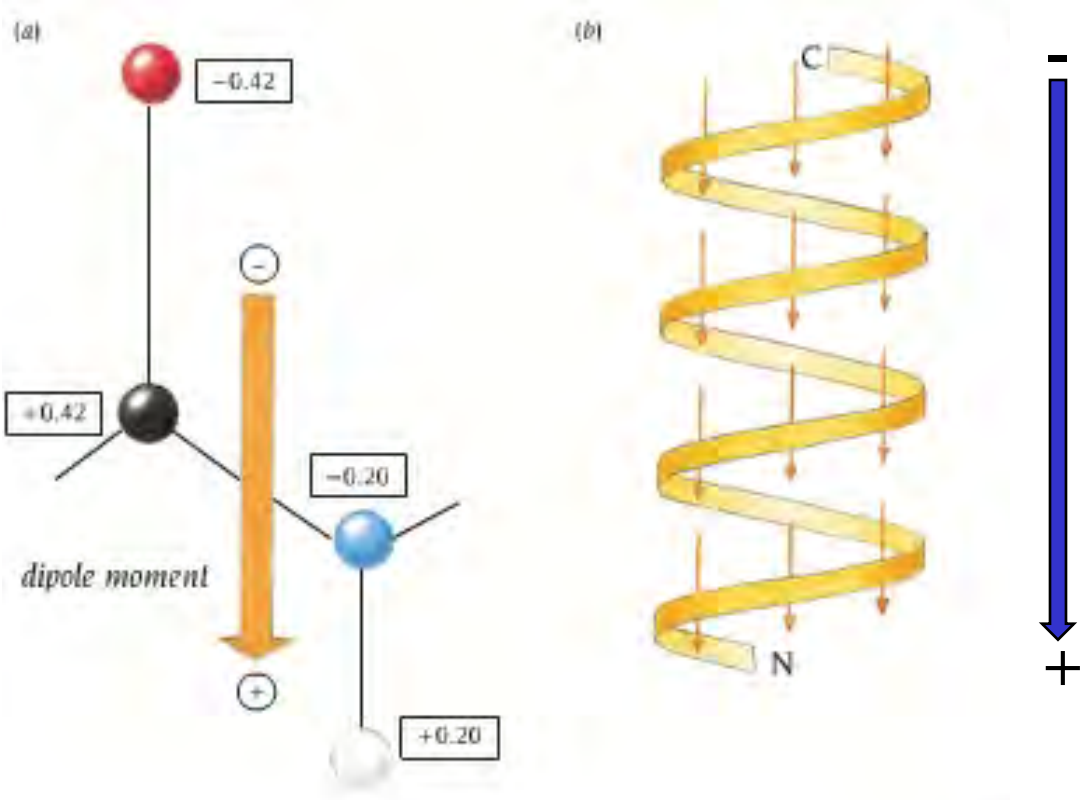


This dipole-dipole interaction is attractive because the positive end of the right dipole is close to the negative end of the left dipole ($U \alpha 1/r^3$). This close attractive interaction dominates the overall energy of the interaction, but there is charge repulsion as well.



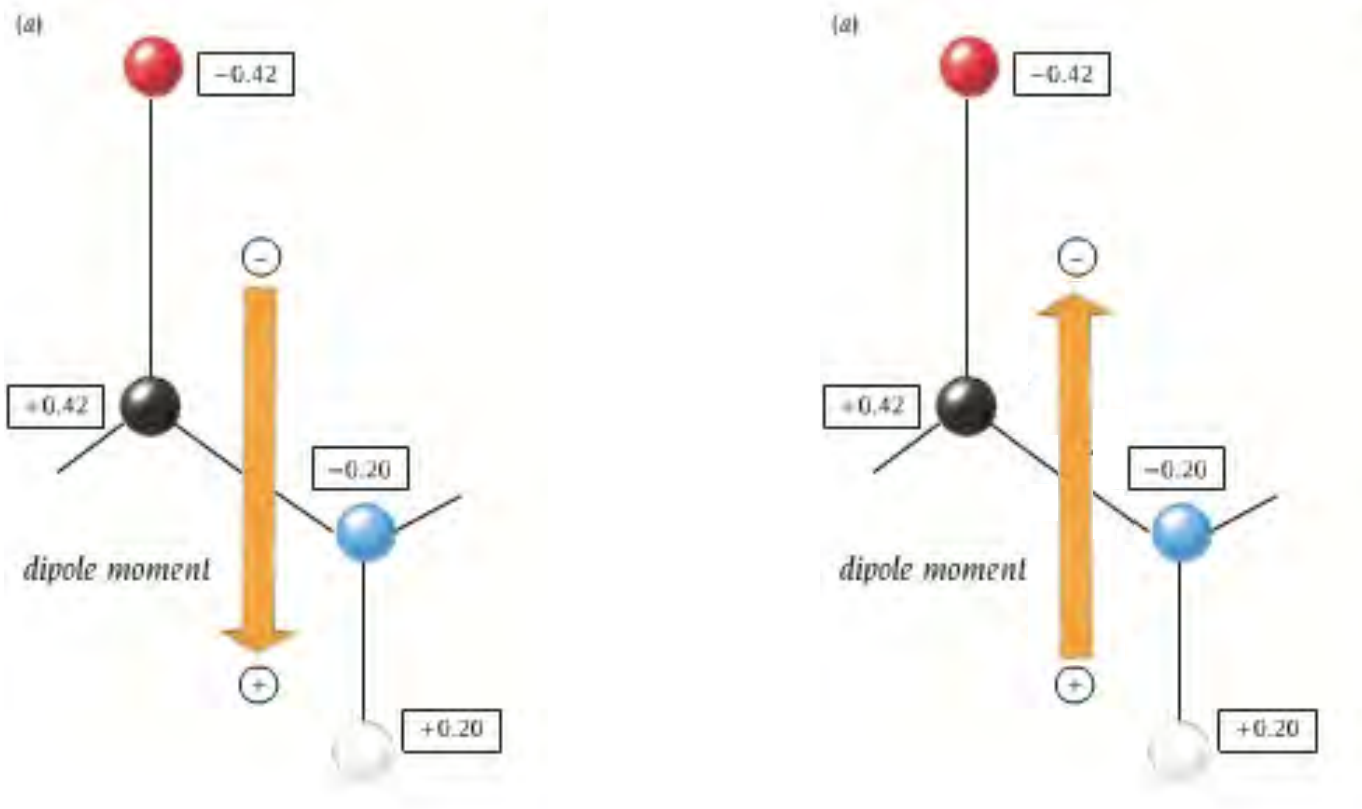
These schematic diagrams summarize the orientation dependence of dipole-dipole interactions

Dipole-dipole interactions in an α-helix



In an α-helix, the individual dipoles of each peptide bond are oriented in the same direction. Dipole moments are additive, so they add together to give a large dipole moment for the entire α-helix, which gives the N-terminus a positive charge, which can interact with negatively charged ligands.

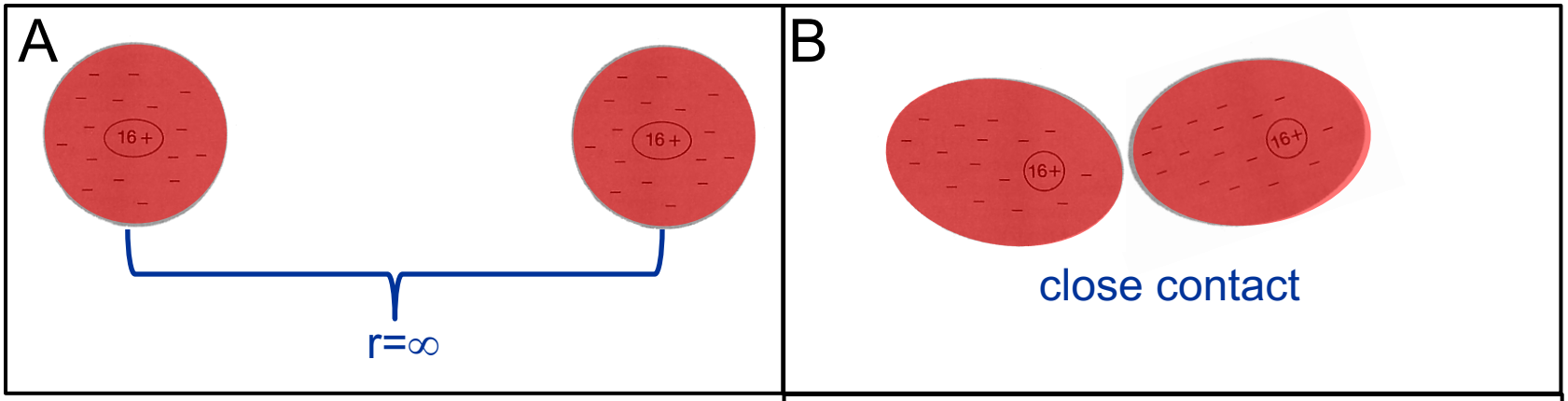
The direction of a dipole moment?



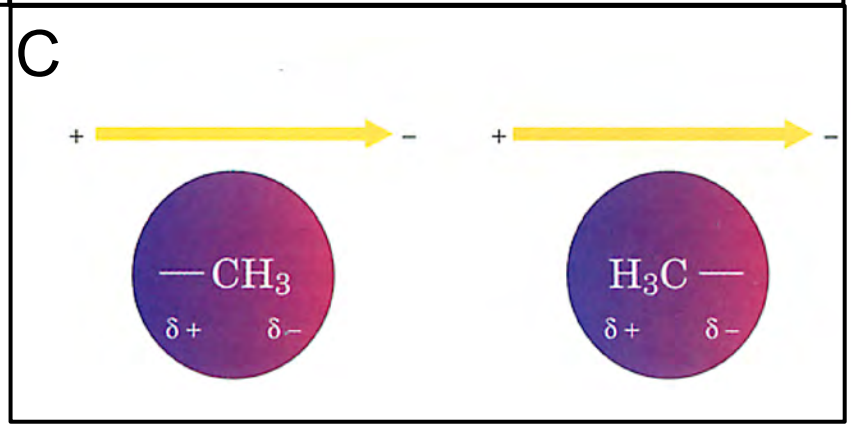
Different texts use different conventions to draw the direction of a dipole moment – i.e from negative to positive (left) or positive to negative (right). Here we *use the positive to negative convention on the right where possible.* You can draw the dipole moment either way, simply define the positive and negative poles of the dipole!

London dispersion: (induced dipole:induced dipole)

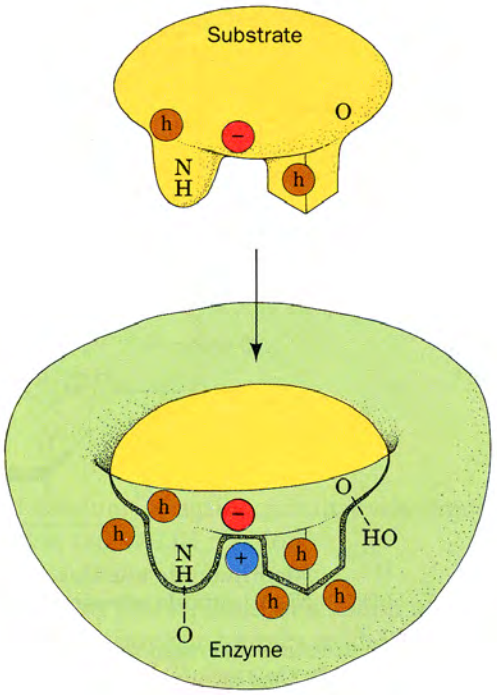
London dispersion forces are very weak attractive interactions between two molecules with no net charge distribution:



London dispersion forces are common between aliphatic side chains, such as between the methyl groups of alanine *(C).*



Molecular interactions are governed by:

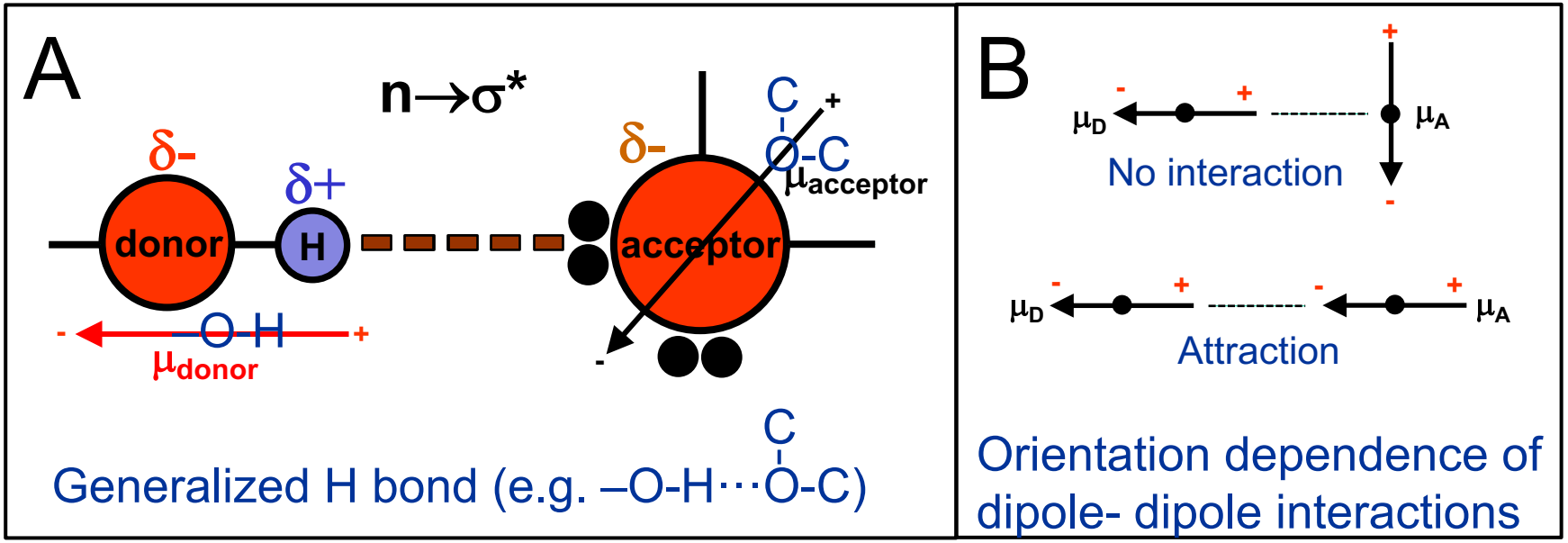


A schematic diagram of the forces that govern protein-ligand interactions.

- steric repulsion
- electrostatic (salt bridge, charge-charge repulsion, van der Waals)
- ***hydrogen bonding***
- hydrophobic (h)

Hydrogen bond

Hydrogen bonds are attractive interactions between a weakly acidic proton donor and a proton acceptor with a free pair of electrons:

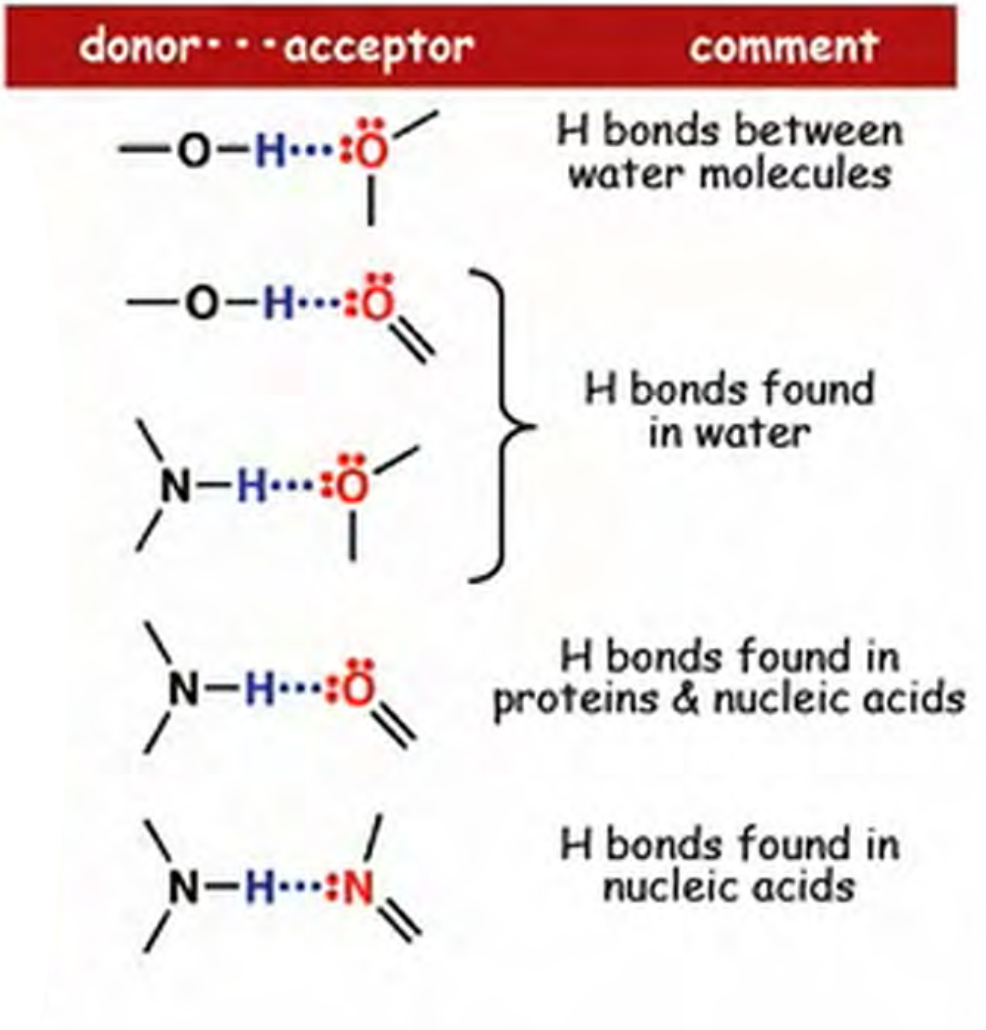


(*A*) Hydrogen bonds have both an electrostatic and a covalent component. The electrostatic component arises from interactions between the dipoles of the donor and acceptor, but the orientation of these is not optimal (*B*). The optimal H bond orientation maximizes the covalent contribution to the bond – i.e. it aligns and maximizes interactions between the n (acceptor) and σ* (donor) orbitals.

Hydrogen bond

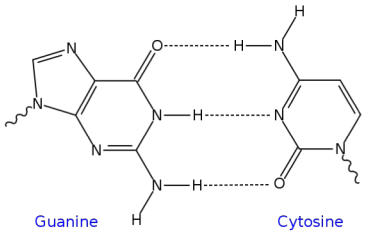
Many different hydrogen bonds are found in biological systems. The optimal distance between the acceptor and donor atoms is **2.7 to 3.1** Å. The interaction energy of a hydrogen bond in a vacuum is roughly **-12 to -30 kJmol^{-1}**.

See:
http://www.youtube.com/watch?v=bh49-A4OEzI



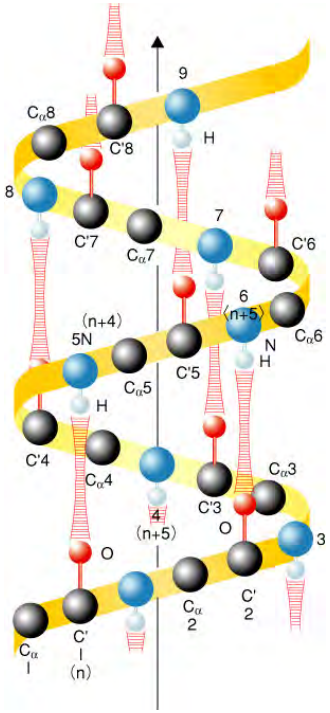
Hydrogen bonds in biology

A



Hbonds in the DNA double helix

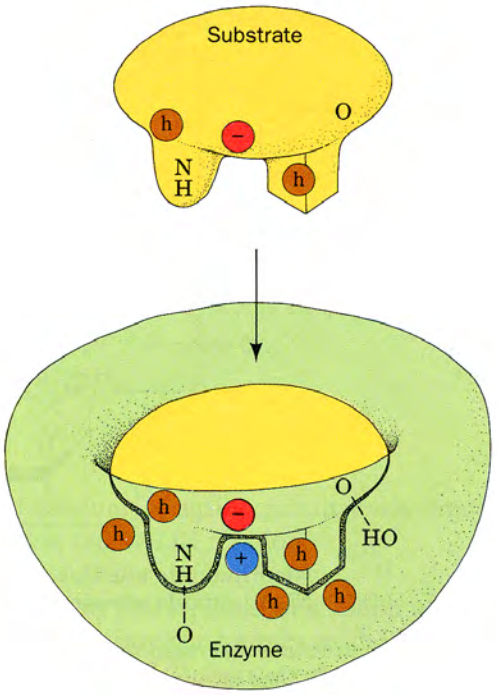
B



H bonds in a protein α-helix

H bonds are dominant features between the stacked base pairs in a DNA double helix *(A)* and between backbone amide groups in an α-helix *(B)*. In fact, all structures and interactions form to maximize H-bonds!

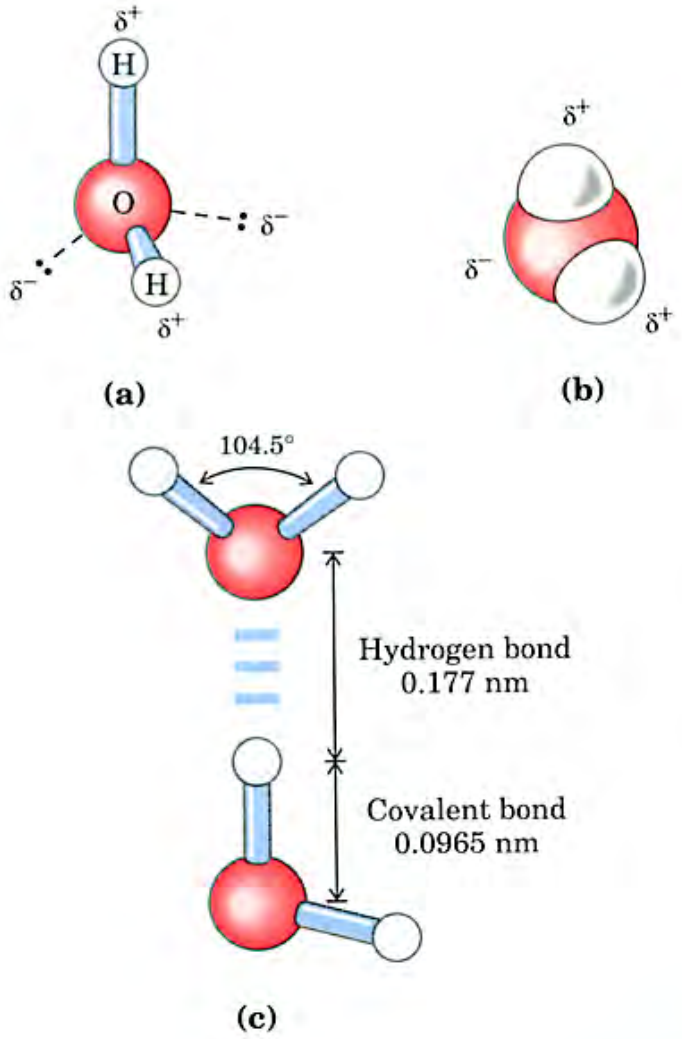
Molecular interactions are governed by:



A schematic diagram of the forces that govern protein-ligand interactions.

- steric repulsion
- electrostatic (salt bridges, charge-charge repulsion, van der Waals)
- hydrogen bond
- ***hydrophobic (h)***

Hydrophobic effect: consider water as a solvent



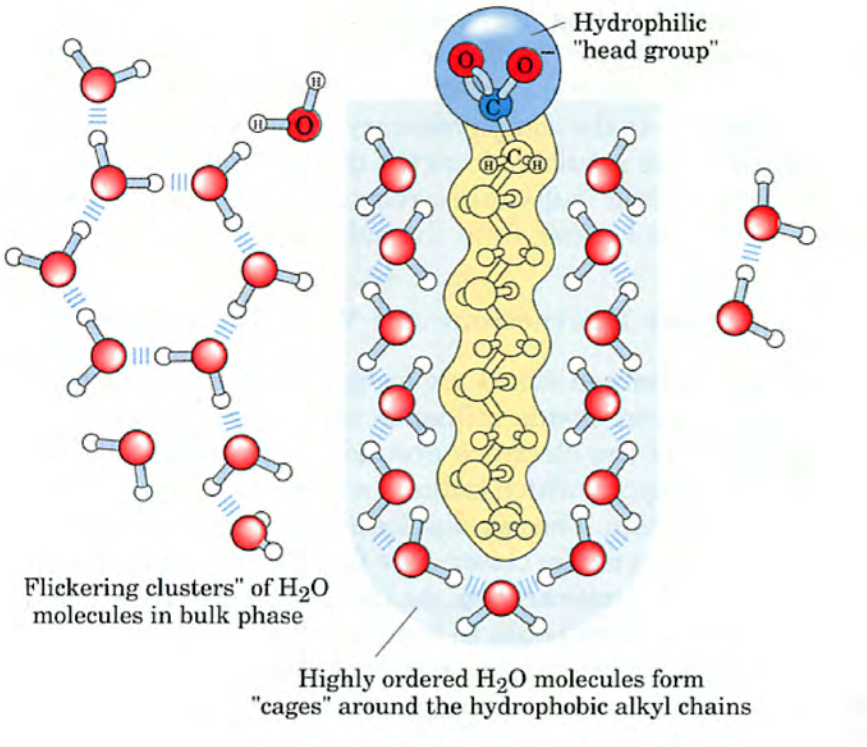
Water is a unique solvent because of its hydrogen bonding capacity. Each water molecule can act as a hydrogen bond donor for 2 hydrogen bonds, and a hydrogen bond acceptor of two hydrogen bonds.

One molecule can therefore form 4 hydrogen bonds!

Hydrophobic molecules disrupt H-bonding

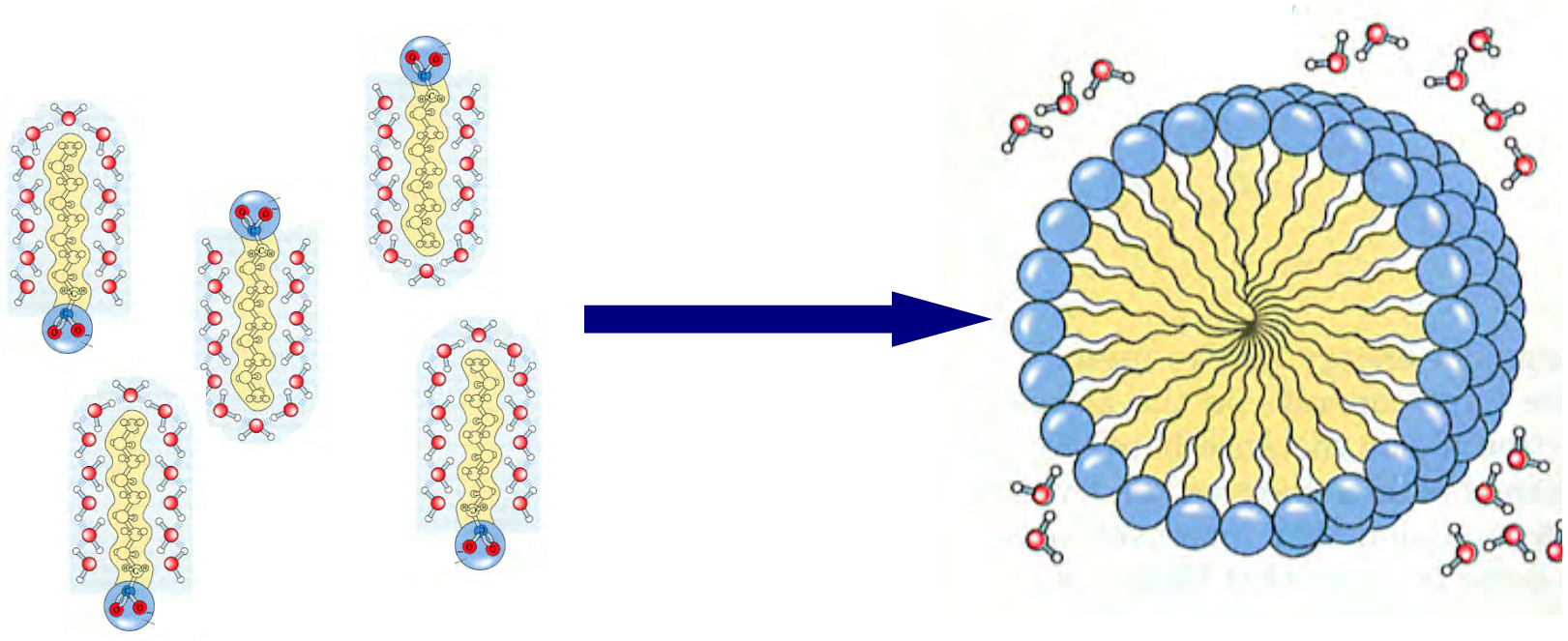
A **hydrophobic molecule**, such as a lipid or an aliphatic side chain disrupts H bonds between water molecules. The disruption of H bonding is not favorable – especially since they are replaced with weak diople-induced dipole interactions.

So the water tends to form an ordered cage around the hydrophobic entity, which minimizes the disruption of H bonds (i.e. maximizes H bonds between water molecules).



Hydrophobic molecules tend to clump together in order to minimize their surface exposure to water.

Hydrophobic effect



Individual hydrophobic lipids must be solvated with a large number of *ordered* water molecules – leading to a large unfavorable entropy

A lipid aggregate (micelle) has less exposure of hydrophobic groups to water, induces less ordering of water, and is entropically favored

Hydrophobic effect

The hydrophobic effect is mainly an entropy driven process (in contrast to electrostatic interactions which are enthalpic in nature) – i.e. it is not the electrostatic interactions between non-polar molecules that drives them together – it is the thermodynamic energy gained by minimizing the ordering of water *(the whole system!)*.

The clumping of hydrophobic molecules together also minimizes the disruption of hydrogen bonding between water molecules (i.e. maximizes inter-water hydrogen bonds).

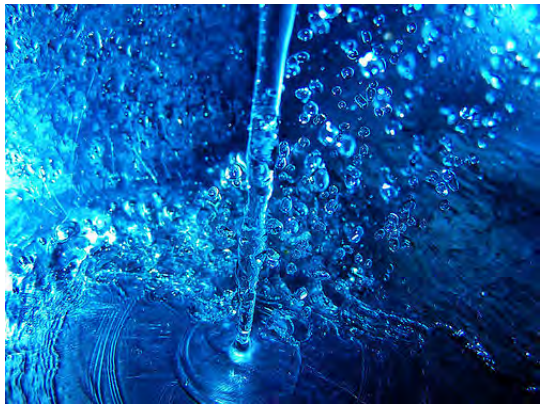
The hydrophobic effect is a major force that drives the folding of proteins, the formation of protein-ligand interactions, the formation of lipid bilayers, etc.

Summary of interaction energies

Type	Interaction Energy (kJ mol^{-1})
Covalent Bond	-200 to -800
Ionic Bond (salt bridge)	±40 to ±400
Hydrophobic	-10 to -30
Hydrogen Bond	-12 to -30
London dispersion	-3 to -10*
Ion-dipole	±3 to ±10
Dipole-dipole	-0.5 - -3
Dipole-induced dipole	-0.4 to -3
Ion-induced dipole	-0.4 to -3
Thermal energy kT** at 25 ^{o}C	2.5

*individual London dispersion interactions are weak, but there are many in a protein and they provide substantial energy; **thermal energy =Boltzmann constant (k) x Temperature (K) x N_A. *Energies are for a vacuum!*

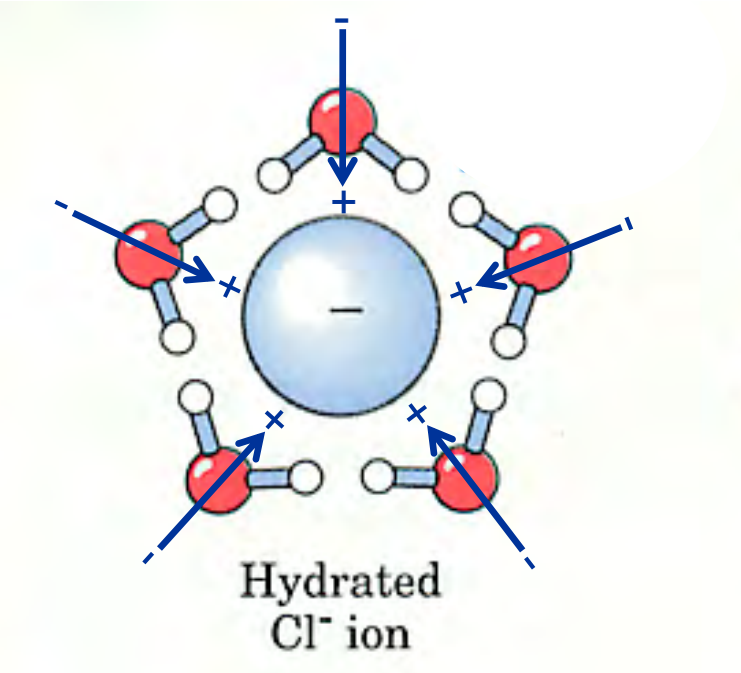
Role of water in protein function

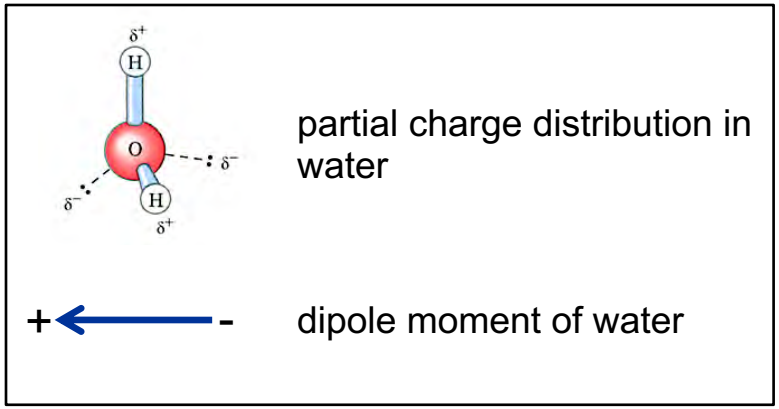





Water nourishes us and makes life possible, it also has a tremendous effect on the mechanisms by which biological molecules interact with each other!

The charged molecule is solvated by water

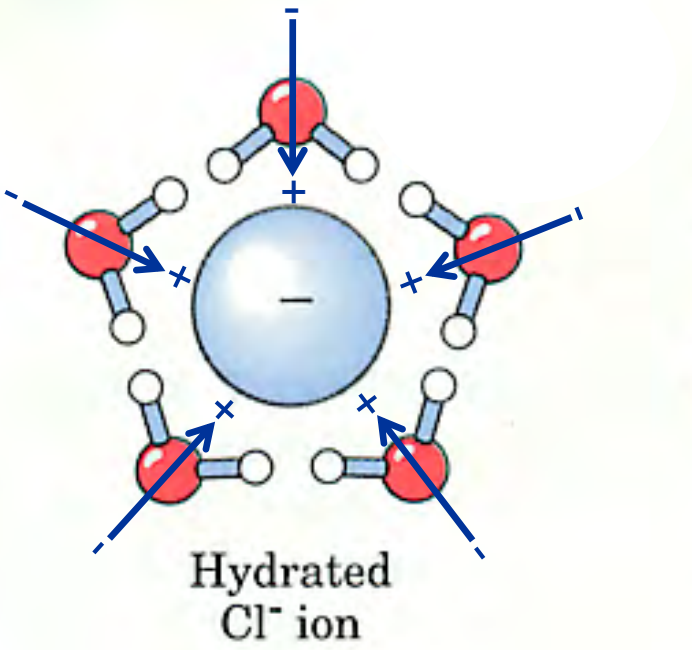




Here we see that water forms an ordered solvation shell to maximize both H-bonding between water molecules and charge-dipole interactions between the Cl^- ion and the dipole moment of water. The charge-dipole interactions are strong and favorable. They strongly counteract the entropically unfavorable ordering of water.

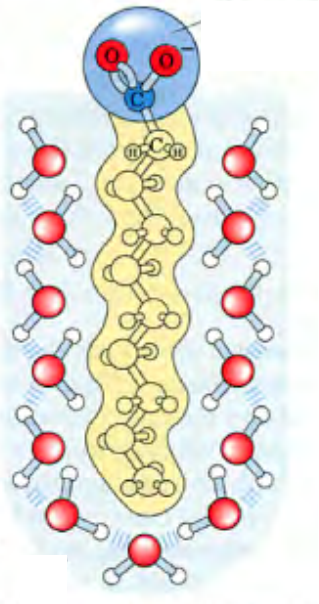
Solvation of charged vs hydrophobic molecules

A



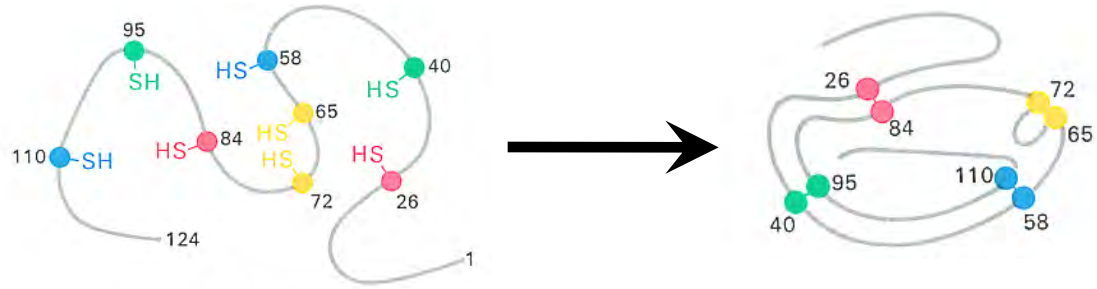
The solvation of an ion (or a polar hydrogen bonding group) is favorable because the formed charge-dipole interactions make up for both the enthalpic loss of H-bonding between water molecules and the unfavorable entropic ordering of water.

B



The solvation of a lipid (or other hydrophobic molecule) is unfavorable because the weak dipole-induced dipole interactions between water and the lipid do NOT make up for both the enthalpic loss of H-bonding between water molecules and the unfavorable entropic ordering of water

Consider the energetics of protein folding



Unfolded

Folded

Consider the folding of a hypothetical 100 amino acid protein:

Our back of the envelope calculation suggests that folded proteins should be rock solid, but in reality, proteins are typically only marginally stable. *Why are protein structures not as stable as predicted by our "back-of-the-envelope" calculation?*

Gibb's free energy

$$A + B \longrightarrow C$$

$$\Delta G_{rxn} = \Delta H - T\Delta S$$

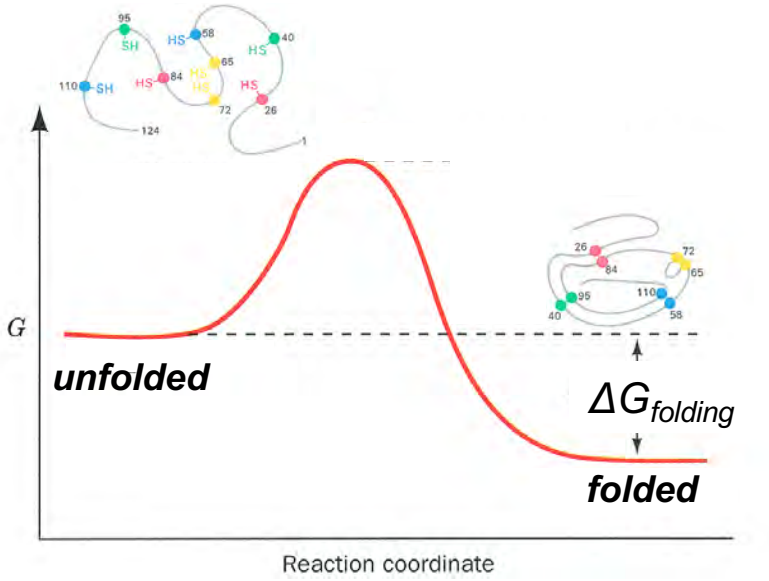
The Gibb's free energy equation governs all biological phenomenon that occur inside a cell. This equation tells us that a reaction or interaction A+B interacting to form C will proceed if the product of the reaction/interaction has a lower energy ($\Delta G_{rxn} < 0$) than the initial reactants.

If $\Delta G_{rxn} < 0$, then the reaction favors the formation of C

For $\Delta G_{rxn} < 0$, the sum of the change in enthalpy and the change in entropy (i.e. the totality of the interactions) must be less than zero.

(ΔH: enthalpy; ΔS: entropy)

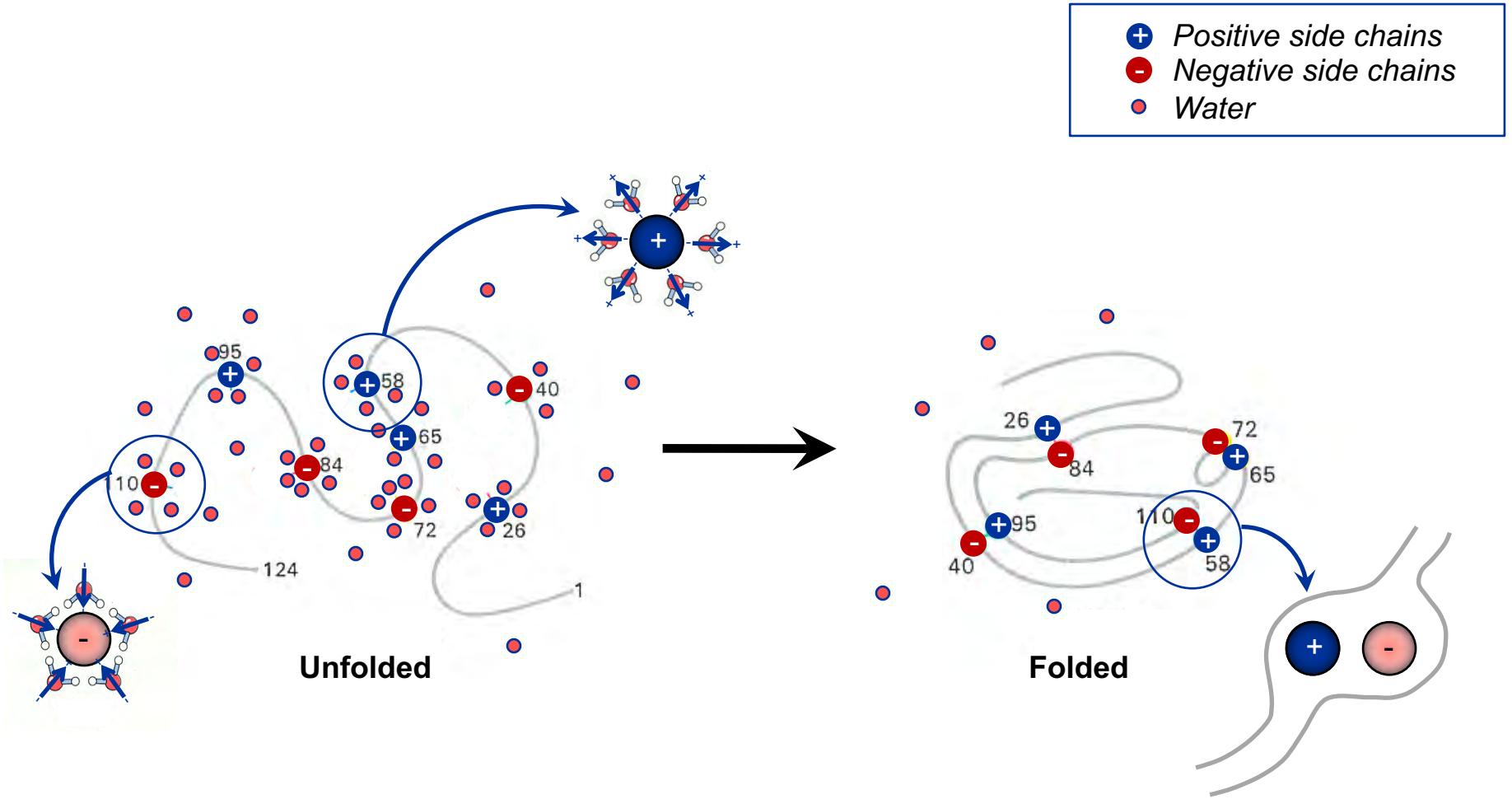
What do we mean by protein stability?



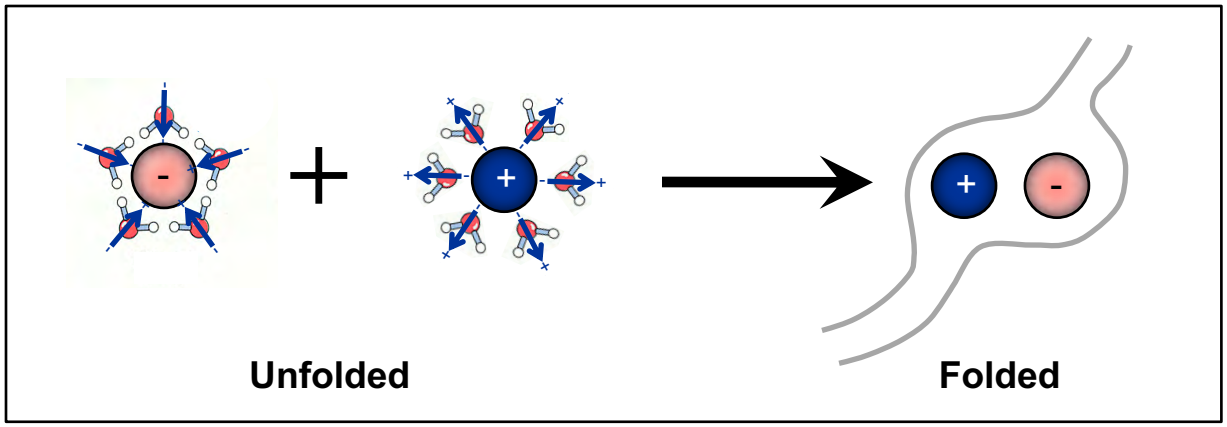
Protein stability refers to $\Delta G_{folding} = G_{folded} - G_{unfolded}$

In our calculation (slide 13), we assumed the energy of the unfolded state is "0" (as in a vacuum). In reality, the unfolded protein is solvated by and interacts with H_2O. We need to understand these solvation energies to understand the energetics of protein folding (*and more generally protein function*)!

Role of ionic interactions in driving folding



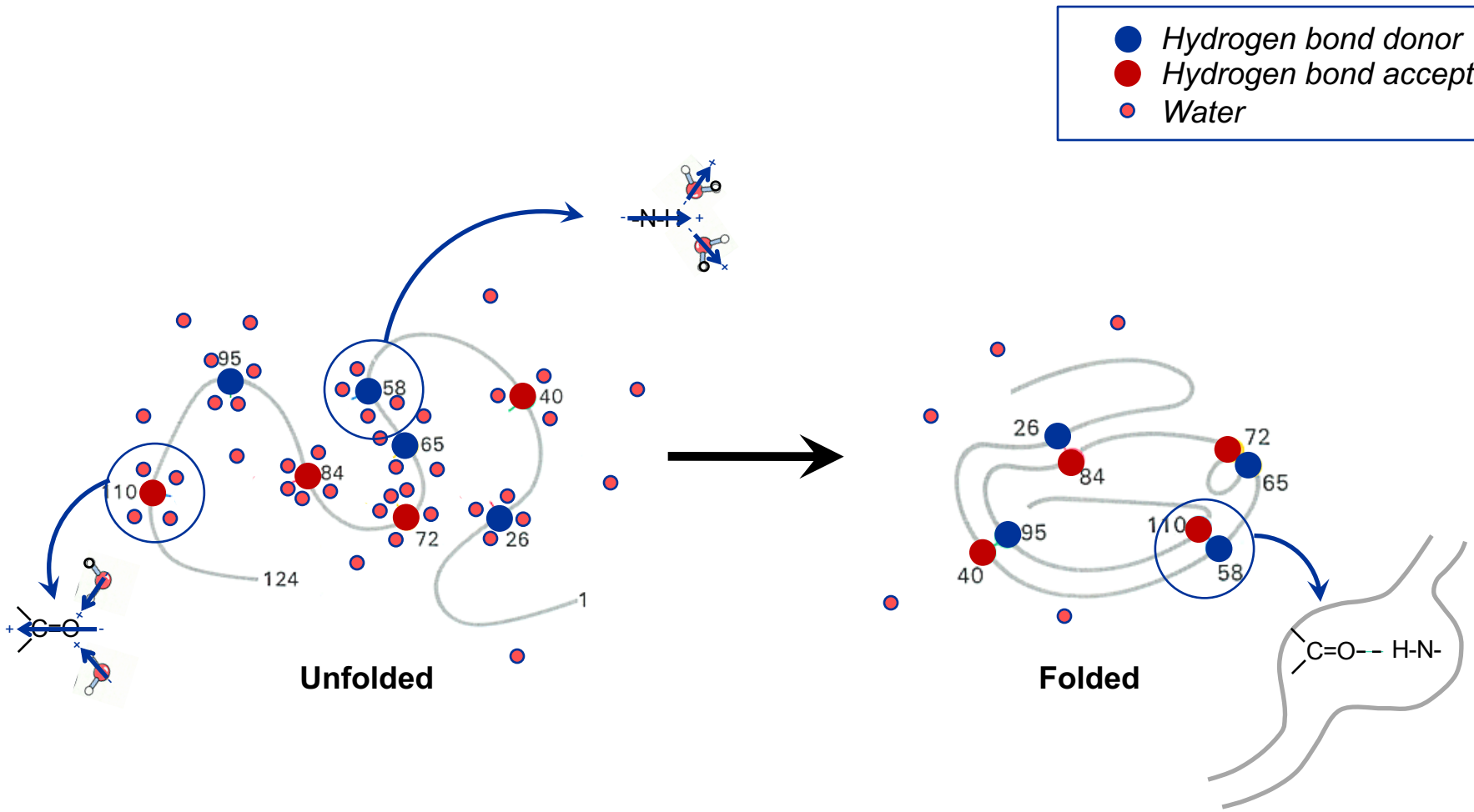
Role of ionic interactions in driving folding



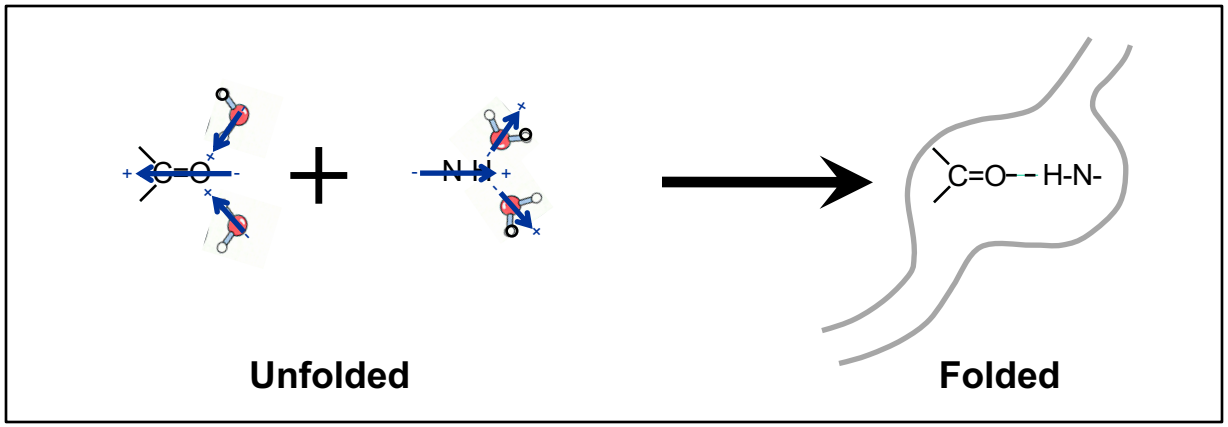
Protein stability $\Delta G_{folding} = G_{folded} - G_{unfolded}$

In the unfolded state, the ionic groups (Lys, Asp, etc.) form charge-dipole interactions with H_2O, so they are quite stable. The energies of the charges solvated by water (unfolded) vs in a salt bridge (folded) are similar - so salt bridges do not contribute a lot of energy to *drive folding* (note that some energy is gained upon folding by releasing the ordered solvating water). *In this context, "energetically driving" refers to contributing substantially to a negative* $\Delta G_{folding}$.

Role of hydrogen bonds in driving folding



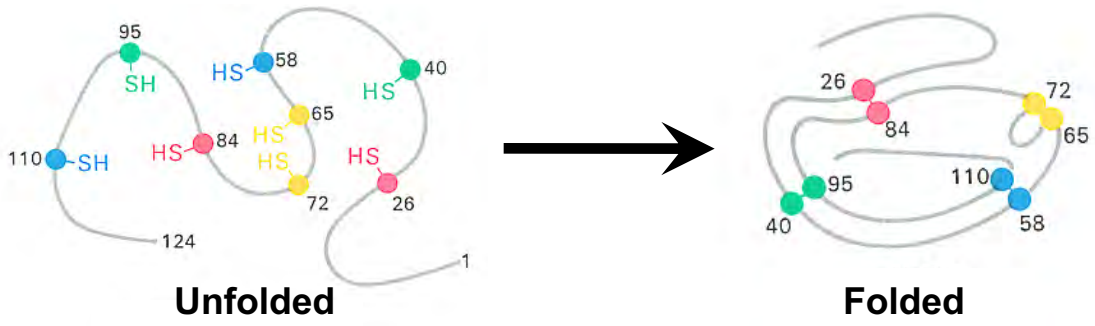
Role of hydrogen bonds in driving folding



Protein stability $\Delta G_{folding} = G_{folded} - G_{unfolded}$

In the unfolded state, the H-bonding groups form H-bonds with H_2O, so they are quite stable. The energies of the groups H-bonded to water (unfolded) vs H-bonded to each other (folded) are similar - so H-bonds do not contribute a lot of energy to drive folding (note that some energy is gained by releasing the solvating water).

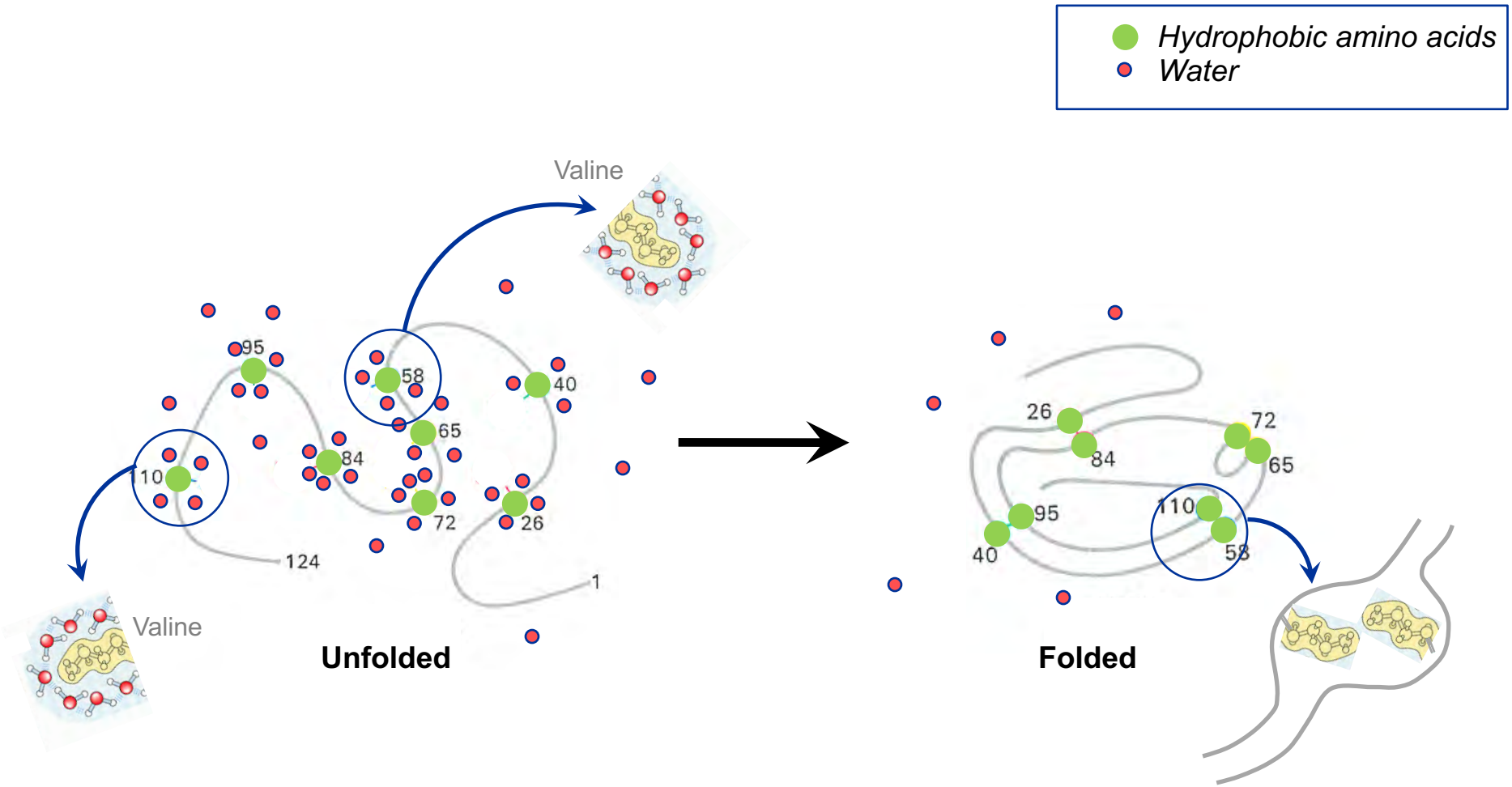
Energetic quandary (dilemma) for protein folding



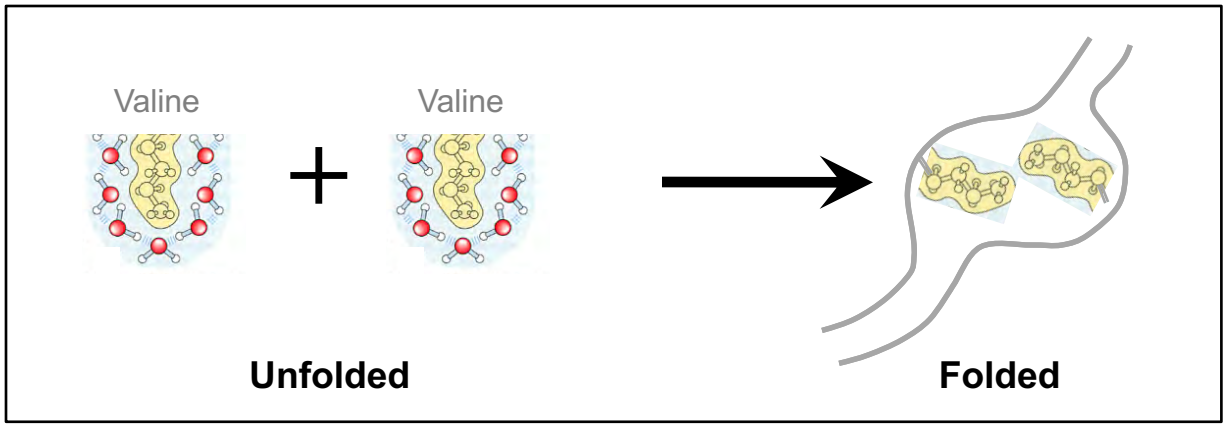
Folded proteins are typically only be 10's of kJ more stable than their unfolded forms despite the plethora of hydrogen bonds and salt bridges. Why are protein structures not more stable?

Minimal stabilization energy is derived from the formation of intramolecular H-bonds or salt bridges, because the H-bonding and ionic groups are just as happy interacting with water in the unfolded state as they are interacting with each other in the folded state. *So, what actually drives protein folding –* ***the hydrophobic effect****!*

Role of the hydrophobic effect



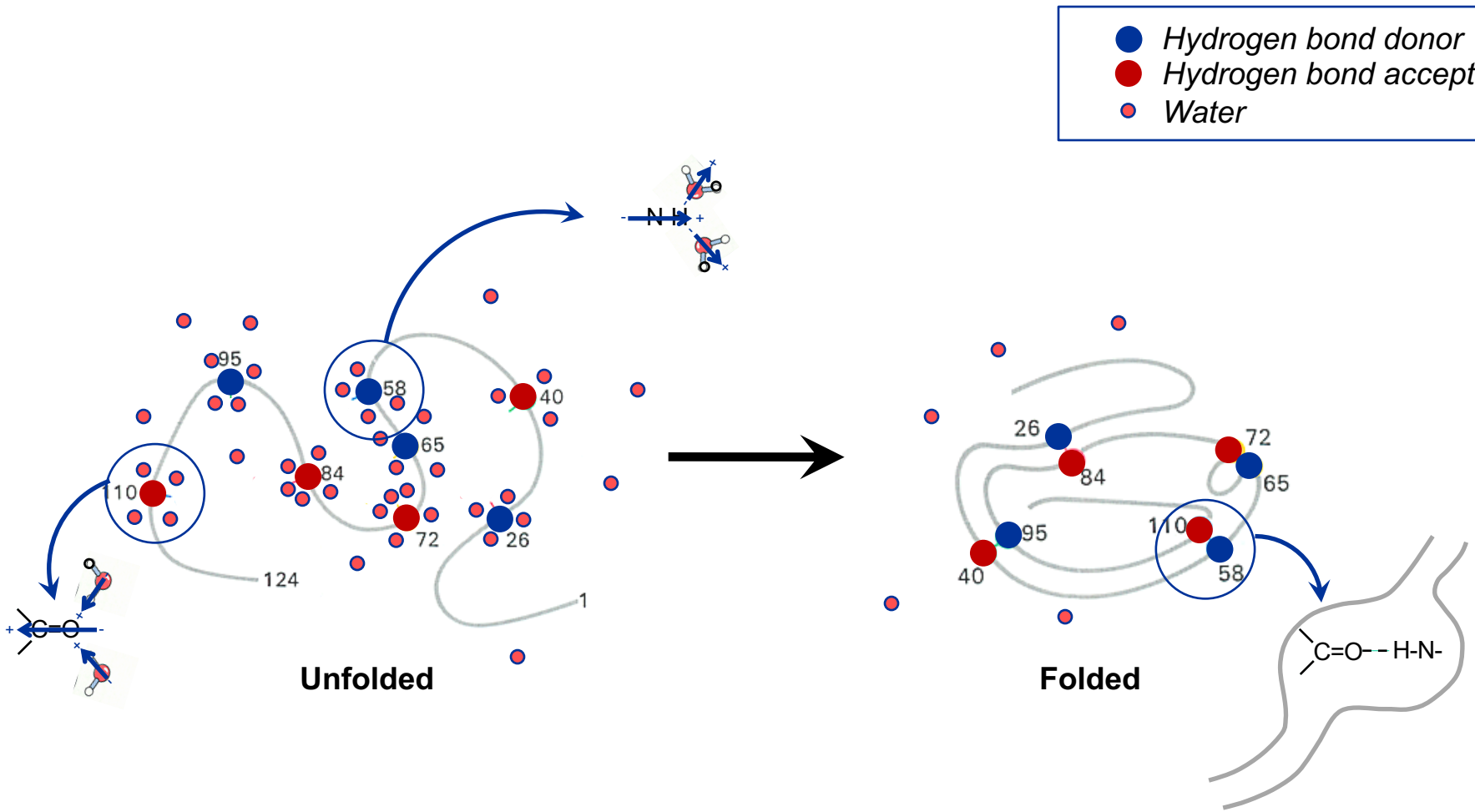
Role of the hydrophobic effect



Protein stability $\Delta G_{folding} = G_{folded} - G_{unfolded}$

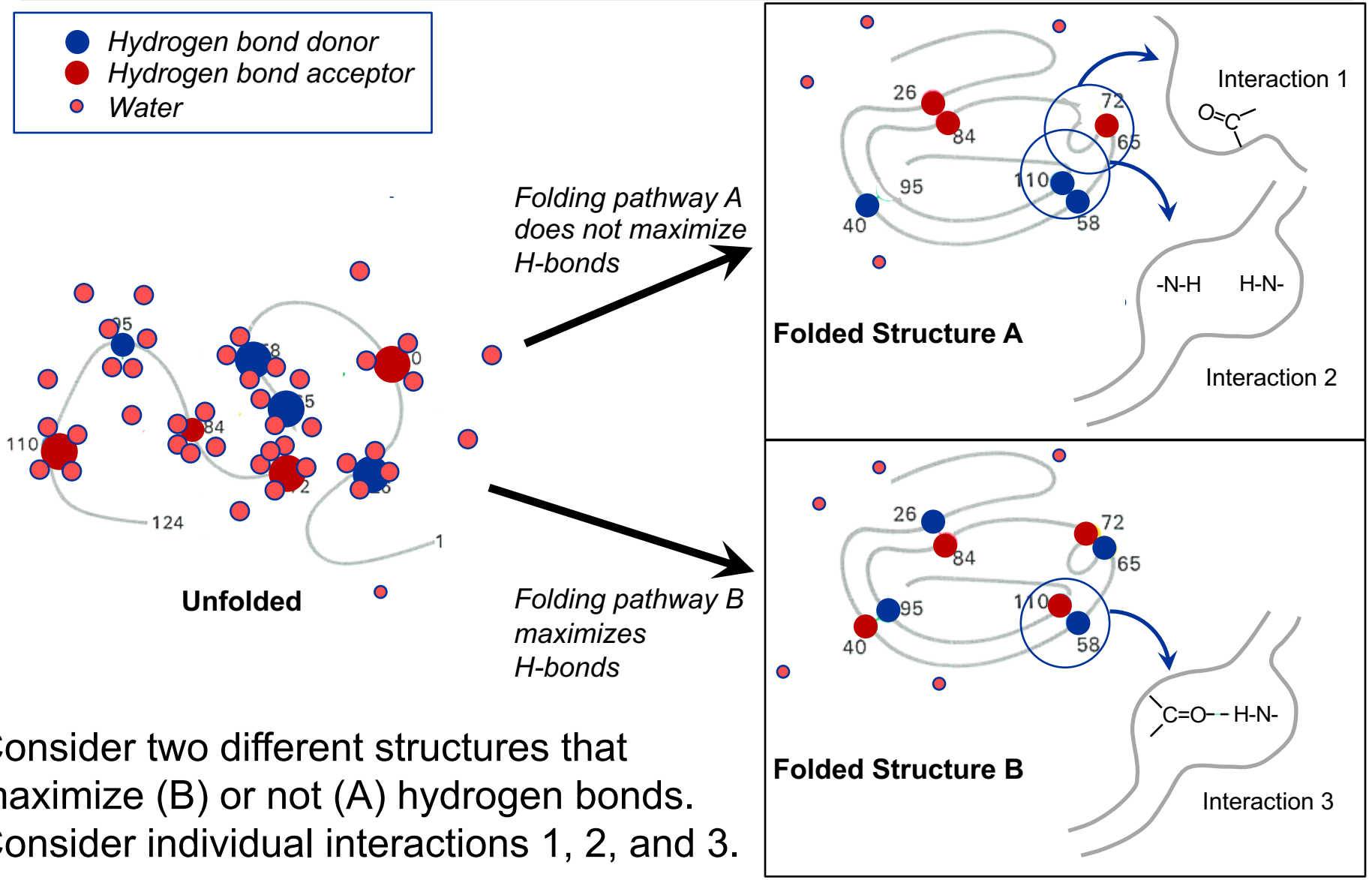
In the unfolded state, hydrophobic groups disrupt H-bonding between H_2O molecules (and cannot replace broken H-bonds with equally favorable interactions). This is very unstable and leads to highly ordered solvating shells. Clumping of the hydrophobic groups on the protein interior releases the ordered water and maximizes H-bonding leading to a large energy gain that drives protein folding.

Role of hydrogen bonds in driving folding



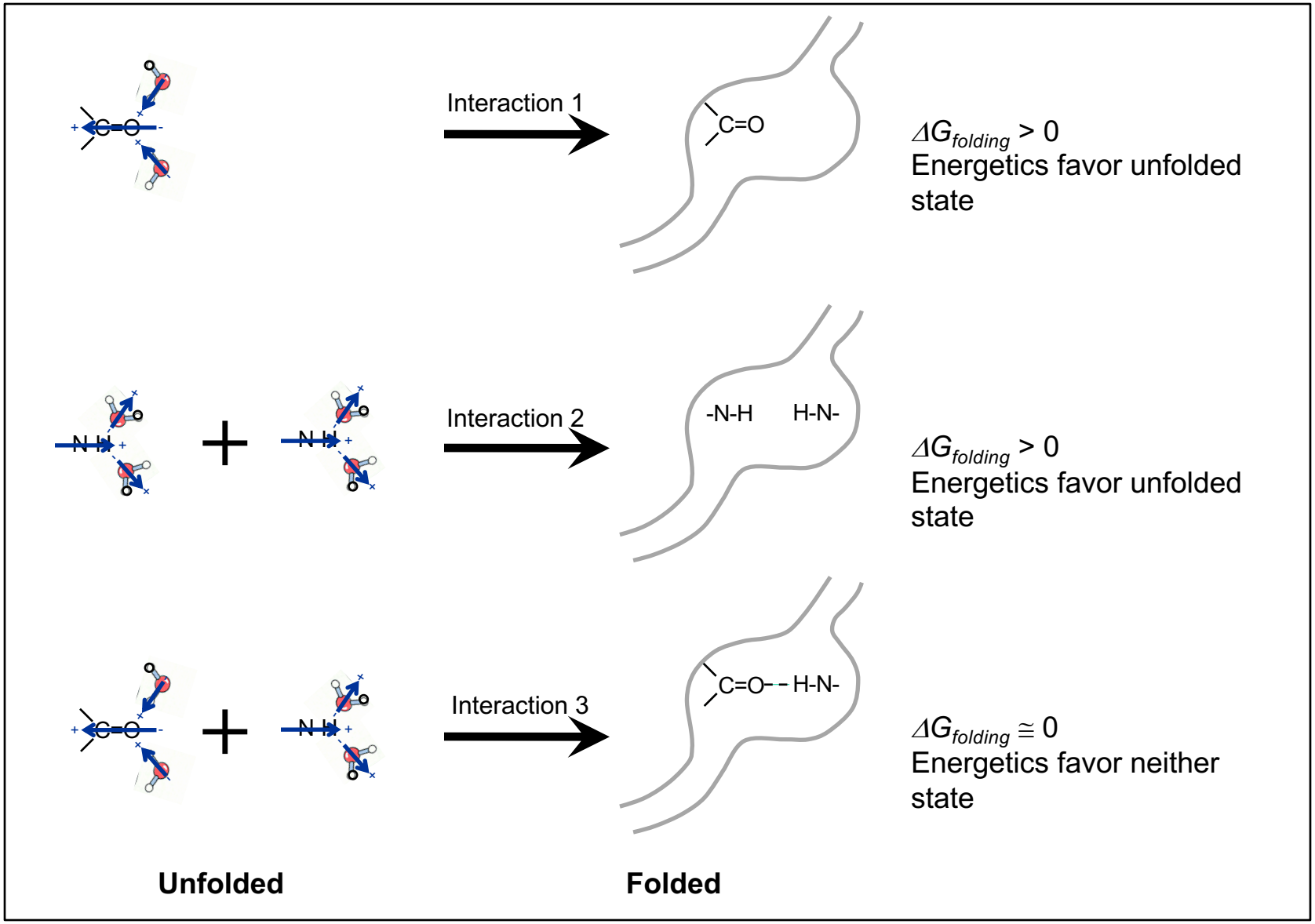
H-bonding does not drive protein folding because H-bonding groups are equivalently stable forming H-bonds with H_2O or with other functional groups in the folded protein structure.

Compare structures were we maximize or not H-bonds



Consider two different structures that maximize (B) or not (A) hydrogen bonds. Consider individual interactions 1, 2, and 3.

Compare interactions that maximize or not H-bonds



Compare structures that maximize or not H-bonds

Protein stability $\Delta G_{folding} = G_{folded} - G_{unfolded}$

Interaction1: H-bonding groups are solvated by H_2O in the unfolded state, but do not form compensating H-bonds in the folded structure. *H-bonding groups are more stable in the unfolded state.*

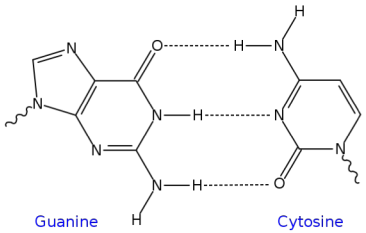
Interaction 2: two H-bonding donors are solvated by H_2O in the unfolded state, but cannot form a compensating H-bond in the folded state. *H-bonding groups are more stable in the unfolded state.*

Interaction 3: H-bonding groups are solvated by H_2O in the unfolded state and form a compensating H-bond in the folded state. *H-bonding groups are equally stable in the unfolded and folded state.*

Folding pathway B leads to a more stable folded structure than folding pathway A. In A, the desolvation energy is not compensated by forming new H bonds (interactions 1 and 2), so these functional groups perfer to be hydrated by water. In B, there is no energetic penalty for de-solvation.

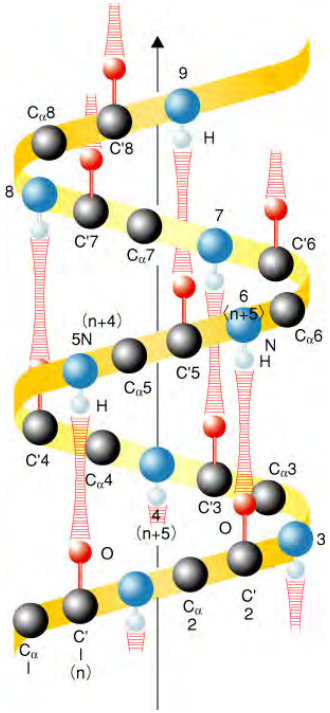
So are H-bonds really important in biology?

A



Hbonds in the DNA double helix

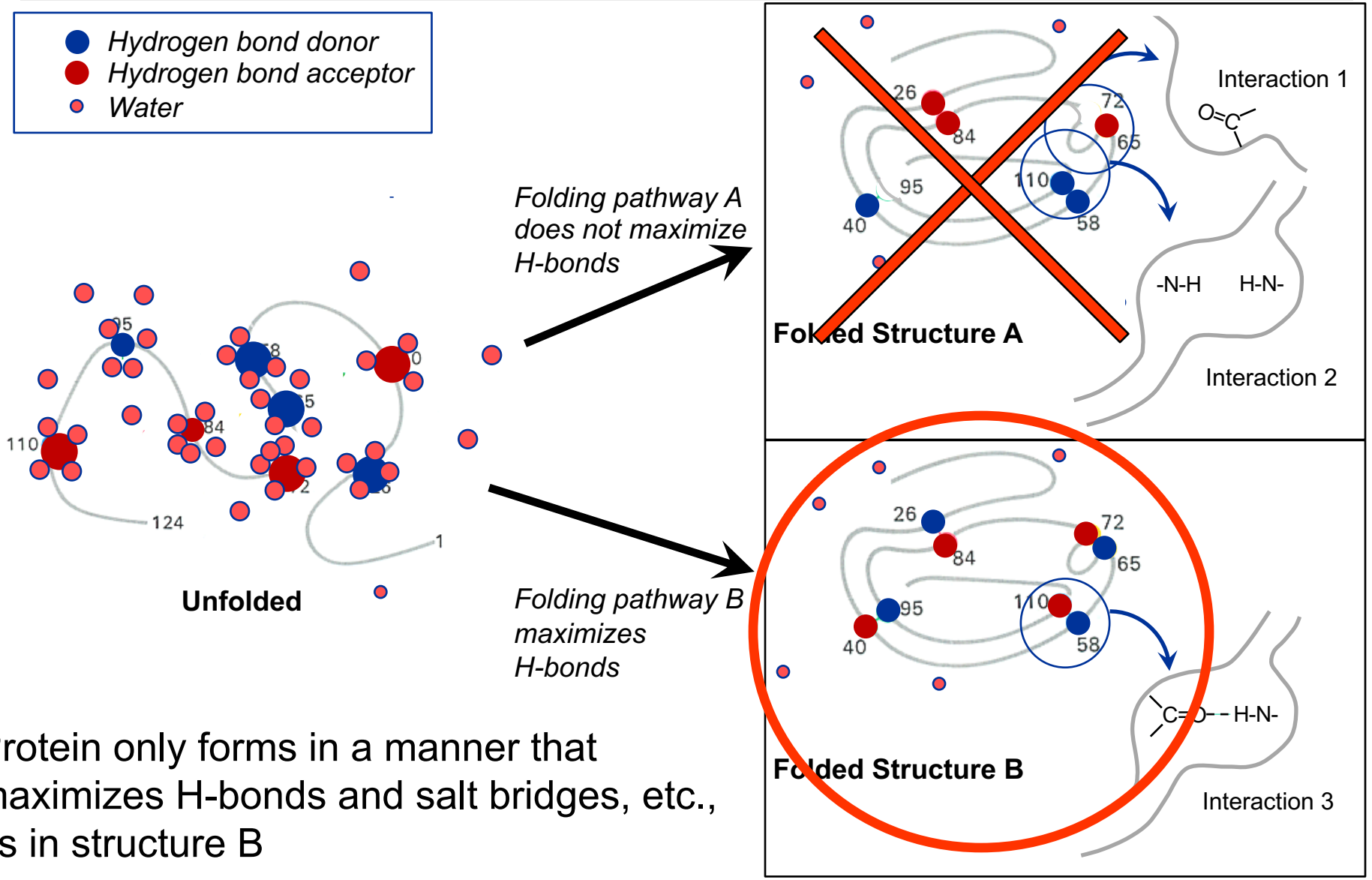
B



H bonds in a protien α-helix

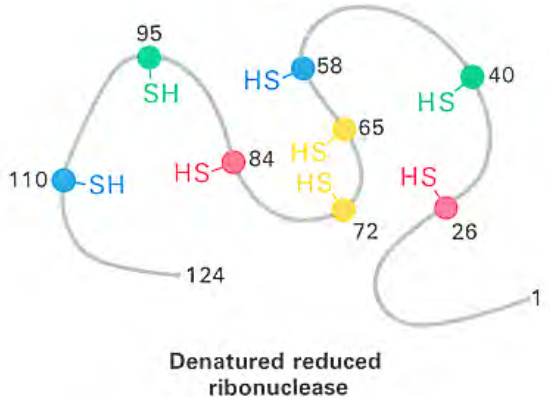
ABSOLUTELY! Salt bridges and H-bonds direct how proteins fold. Proteins fold to maximize salt bridges and H-bonds to compensate for the energy lost due to water desolvation.

Folding is directed to maximize the formation of H-bonds



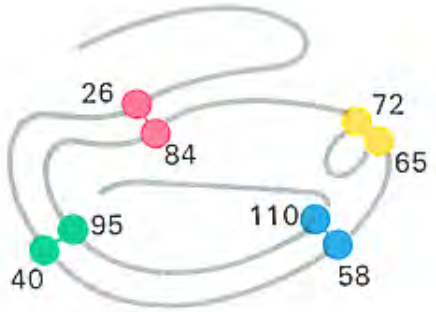
Protein only forms in a manner that maximizes H-bonds and salt bridges, etc., as in structure B

H-bonding plays a key role…

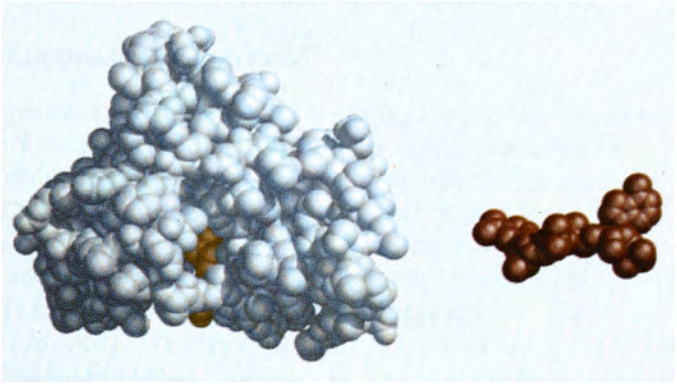


Denatured reduced ribonuclease

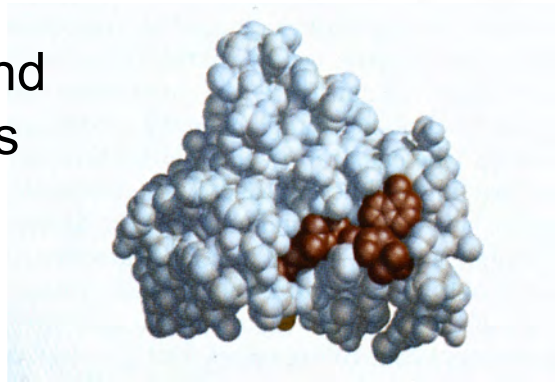
Protein folding



Native ribonuclease



Protein-ligand Interactions



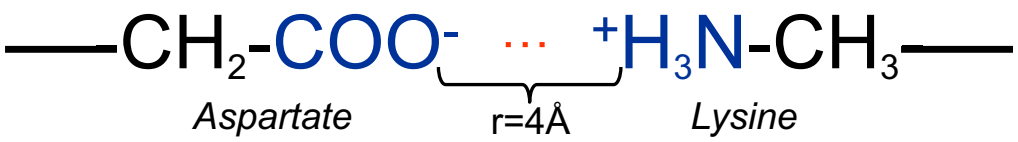
… in all biological interactions directing both protein folding and protein-ligand interactions , etc. H bonding is central to all biological processes.

Remember the thermodynamic hypothesis:

"the three-dimensional structure of a native protein in its normal physiological milieu is the one in which the Gibbs free energy of the *whole system* is lowest; that is, that the native conformation is determined by the *totality of interatomic interactions* and hence by the amino acid sequence, in a given environment."

When dealing with biological phenomenon, the whole system refers to the fact that the aqueous solvent plays a major role in determining biological interactions. We must always consider the effect of water solvation/de-solvation when discussing protein-ligand interactions, protein folding, etc.

Can we account for desolvation energies?



Coulomb's law tells us that the interaction energy between point charges in a vacuum is governed by the magnitude and distance between the charges (*A*). In a solution, the energy of the interaction is also dependent upon the polarity of the solvent, which is a measured by the dielectric constant, *D* (*B*):

A	B
$U = \frac{kq_1q_2}{r}$	$U = \frac{kq_1q_2}{Dr}$
Coulomb's law defines the energy of interaction between two charges in a vacuum (D=1)	*Coulomb's law modified to take into account the effects of solvent polarity, as defined by D (a unit-less parameter)*

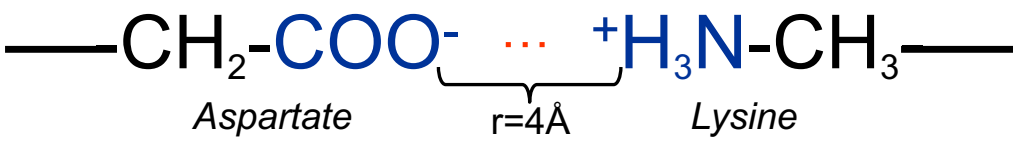
Dielectric constant

Substance	Dielectric Constant	Dipole Moment (debye)
Formamide	110.0	3.37
Water	78.5	1.85
Dimethyl sulfoxide	48.9	3.96
Methanol	32.6	1.66
Ethanol	24.3	1.68
Acetone	20.7	2.72
Ammonia	16.9	1.47
Chloroform	4.8	1.15
Diethyl ether	4.3	1.15
Benzene	2.3	0.00
Carbon tetrachloride	2.2	0.00
Hexane	1.9	0.00

Source: Brey, W.S., *Physical Chemistry and Its Biological Applications*, *p.* 26, Academic Press (1978).

The value of D increases with the polarity of the medium - non-polar solvents have a low D, while **polar aqueous solvents have a D of 80**. In effect, a vacuum has a D=1. The interior of a protein is thought to have low dielectric constants in the range $2 < D < 5$.

Ionic interactions in different environments



Vacuum
D=1

$$U = \frac{(9.0x10^{9}\,JmC^{-2})(1.6x10^{-19}\,C)(-1.6x10^{-19}\,C)}{(1)(4x10^{-10}\,m)} \bullet N_A = -347kJmol^{-1}$$

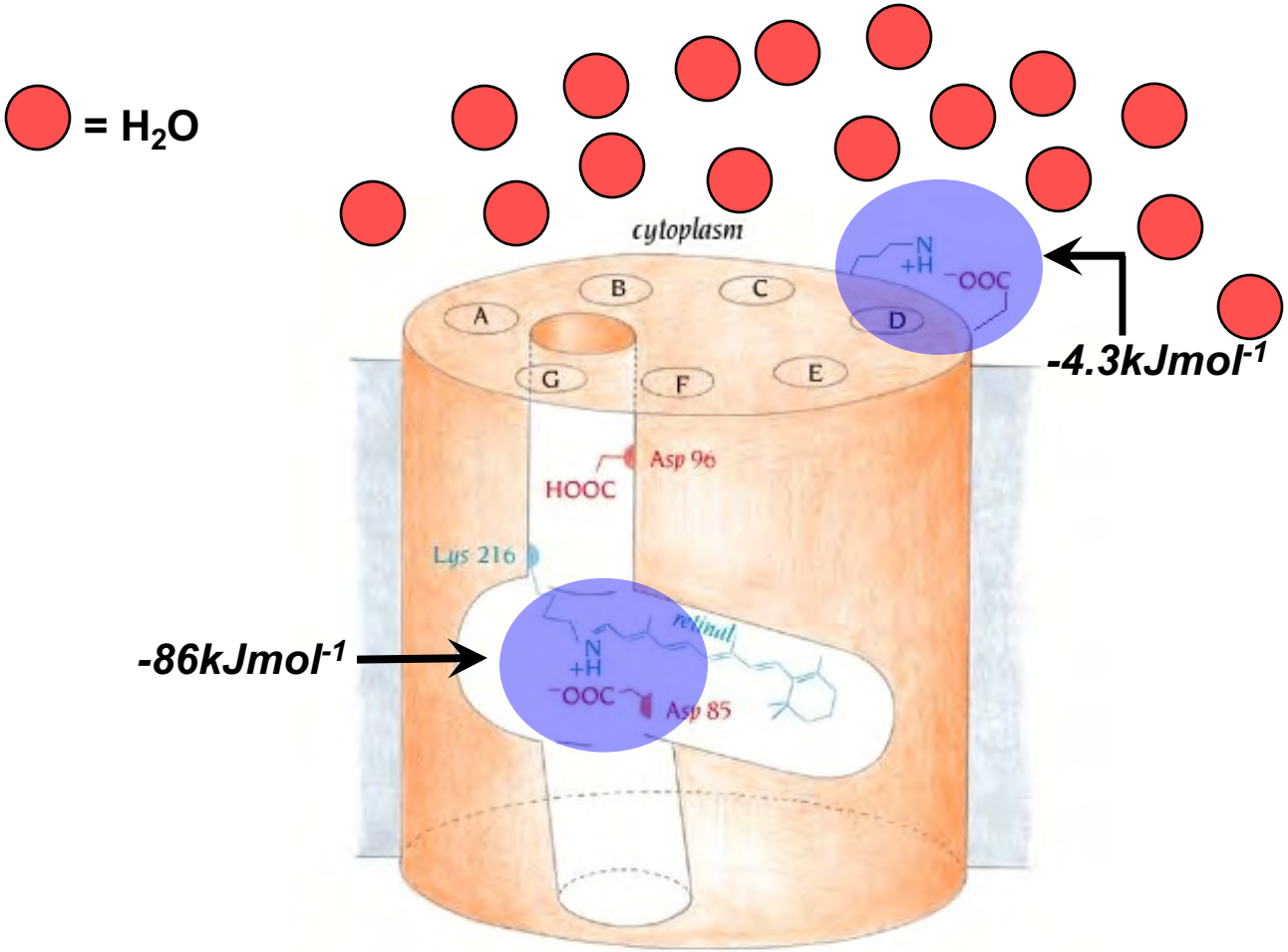
Protein Interior
D= 4

$$U = \frac{(9.0x10^{9}\,JmC^{-2})(1.6x10^{-19}\,C)(-1.6x10^{-19}\,C)}{(4)(4x10^{-10}\,m)} \bullet N_A = -86kJmol^{-1}$$

Protein surface
D=80

$$U = \frac{(9.0x10^{9}\,JmC^{-2})(1.6x10^{-19}\,C)(-1.6x10^{-19}\,C)}{(80)(4x10^{-10}\,m)} \bullet N_A = -4.3kJmol^{-1}$$

Why is energy different inside versus outside?



The salt bridge between D85 and the retinal chromophore of the light receptor, bacteriorhodopsin, (interior of the protein) is 20x stronger than a similar salt bridge exposed to the aqueous environment. *Why?*

Remember this is an energy difference

The interaction energy between two charges separated by 4 Å is the difference in energy between the charges at distance r = 4 Å vs r = ∞.

$$\Delta G_{interaction} = G_{r=4Å} - G_{r=\infty}$$

To define the interaction energy between two molecules, we must define their energies at infinite separation. In a vacuum, the interaction energies of two charges with the environment at infinite separation is essentially zero. In a biological setting, the charges do not interact with each other at r=∞, but they do interact with their "solvent". *So the energy at r=∞ depends on the solvation energy, which is ultimately dependent on the solvent polarity, or its dielectric constant!*

Interaction energy on the interior of a protein

$$\Delta G_{interaction} = G_{r=4Å} - G_{r=\infty}$$

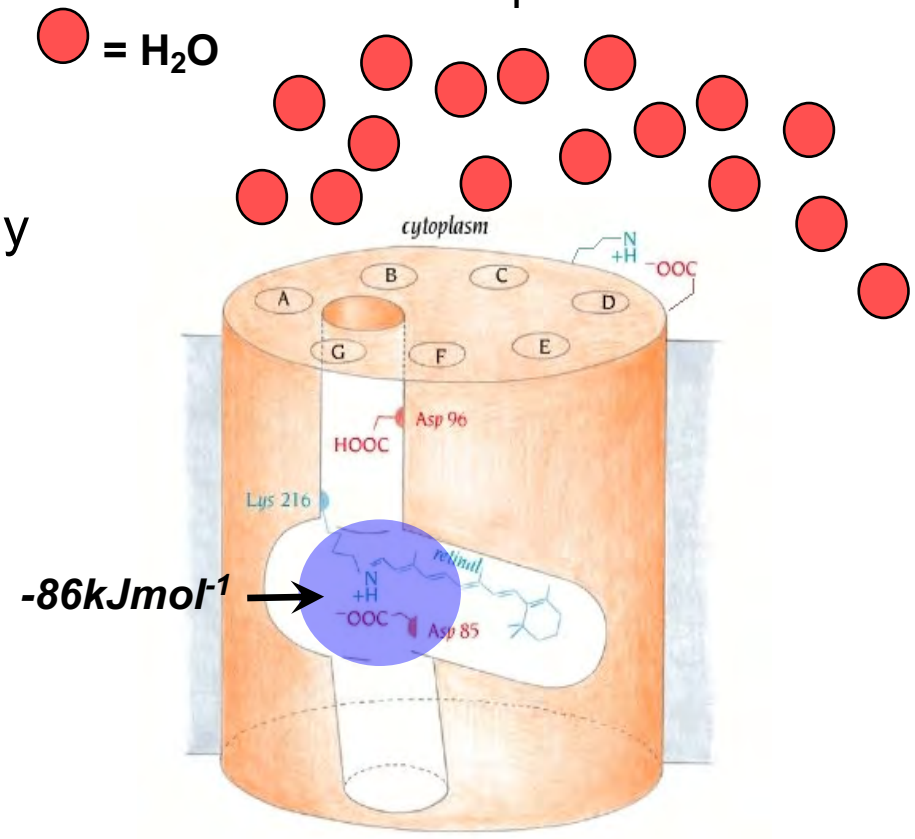
$-CH_2-COO^- \cdots {}^+H_3N-CH_3-$ vs $-CH_2-COO^-$ $^+H_3N-CH_3-$

r=4Å r=∞

= H_2O

On the interior of a protein, the energy of the salt bridge is much stronger than the energy derived from the charges interacting with other non-polar groups of the protein (dipole – induced dipole interactions), so the salt bridge is very strong:

ΔG_{int} is << 0



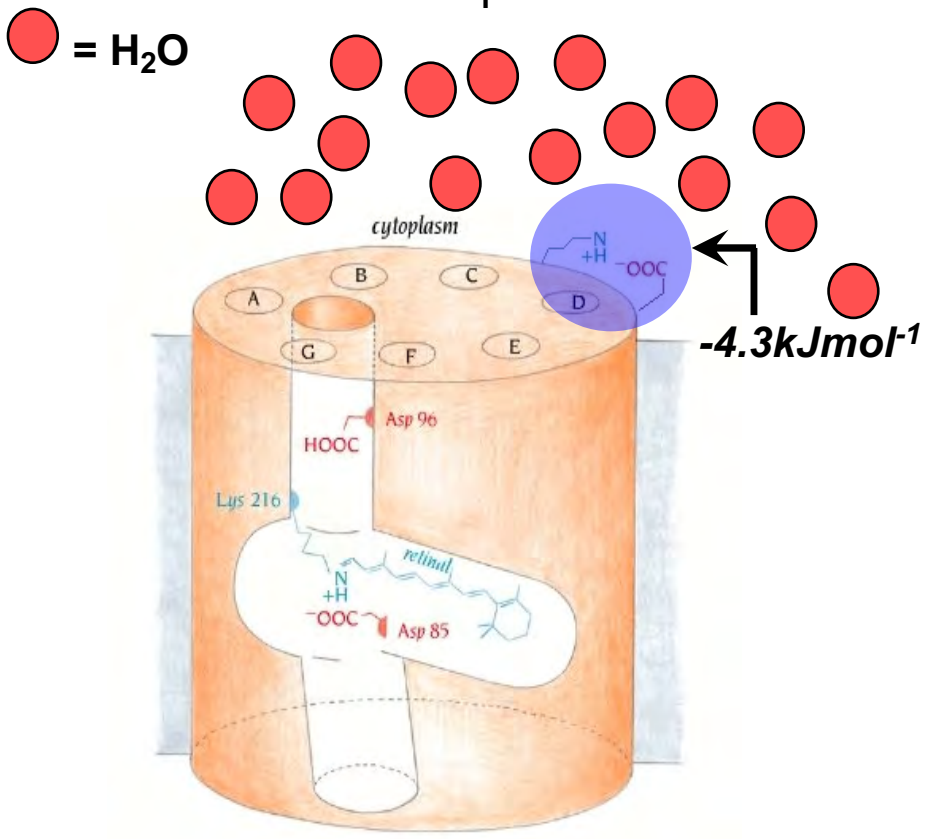
Interaction energy on the protein surface

$$\Delta G_{interaction} = G_{r=4Å} - G_{r=\infty}$$

$-CH_2-COO^- \cdots {}^+H_3N-CH_3-$ vs $-CH_2-COO^-$ $^+H_3N-CH_3-$

r=4Å r=∞

= H_2O



At the protein surface, the charges can form a salt bridge or are solvated by H_2O – where they are quite happy. This is because the energy of solvation is similar to the energy of the salt bridge:

$$\Delta G_{int} \text{ is } \cong 0^*$$

* although we gain some energy from the desolvation of ordered water

In an aqueous environment, individual charges can be solvated by H_2O (at r=∞) or they can form a salt bridge in the folded structure

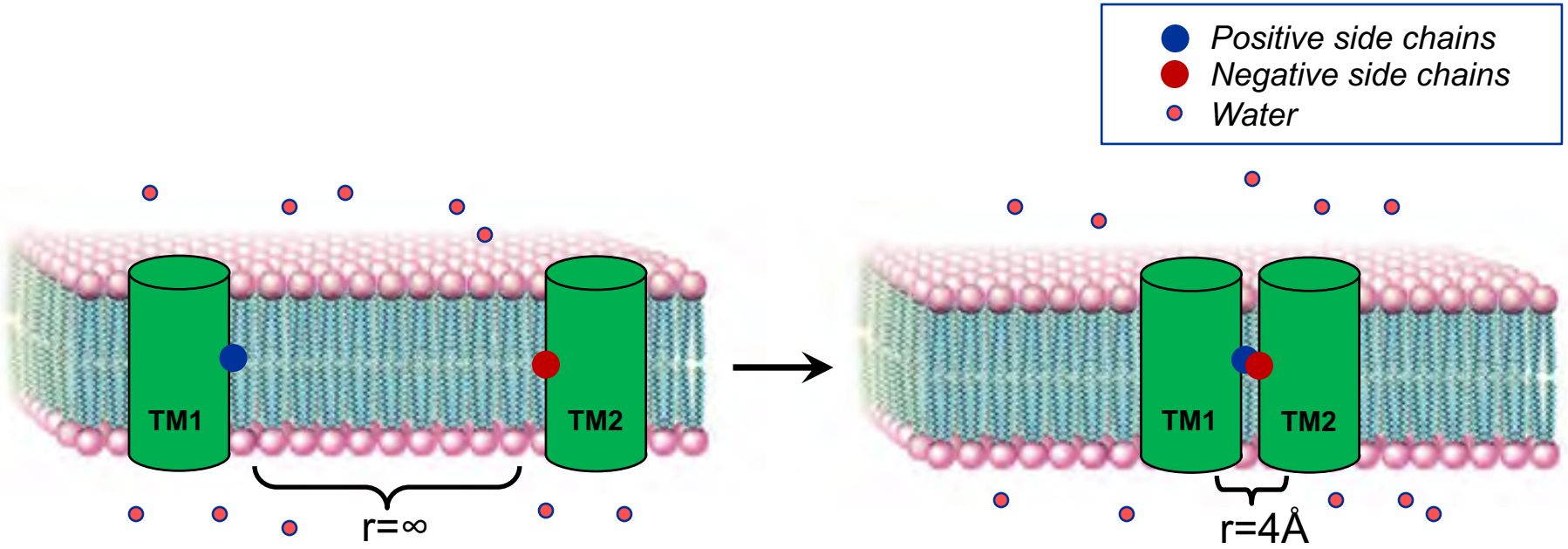
- in terms of enthalpy, the two charges are just as happy to be solvated by water as they are interacting with each other

- a proportion of the energy gained upon forming the salt bridge (-4.3 $kJmol^{-1}$) comes from the entropic gains of desolvating the charges – i.e. releasing the ordered water molecules!

In the hydrophobic interior of the protein, individual charges are not solvated by water at r=∞, they can only interact with the hydrophobic groups typically found in the interior of the protein by weak charge-induced dipole interactions

- the charges are much happier interacting with each other than with the hydrophobic environment, so there is a large enthalpic gain (-86 $kJmol^{-1}$) that occurs upon formation of the salt bridge that drives the two charges together. *Salt bridges and H-bonding can thus provide substantial energy to drive interactions, when they occur in a non-polar environment!*

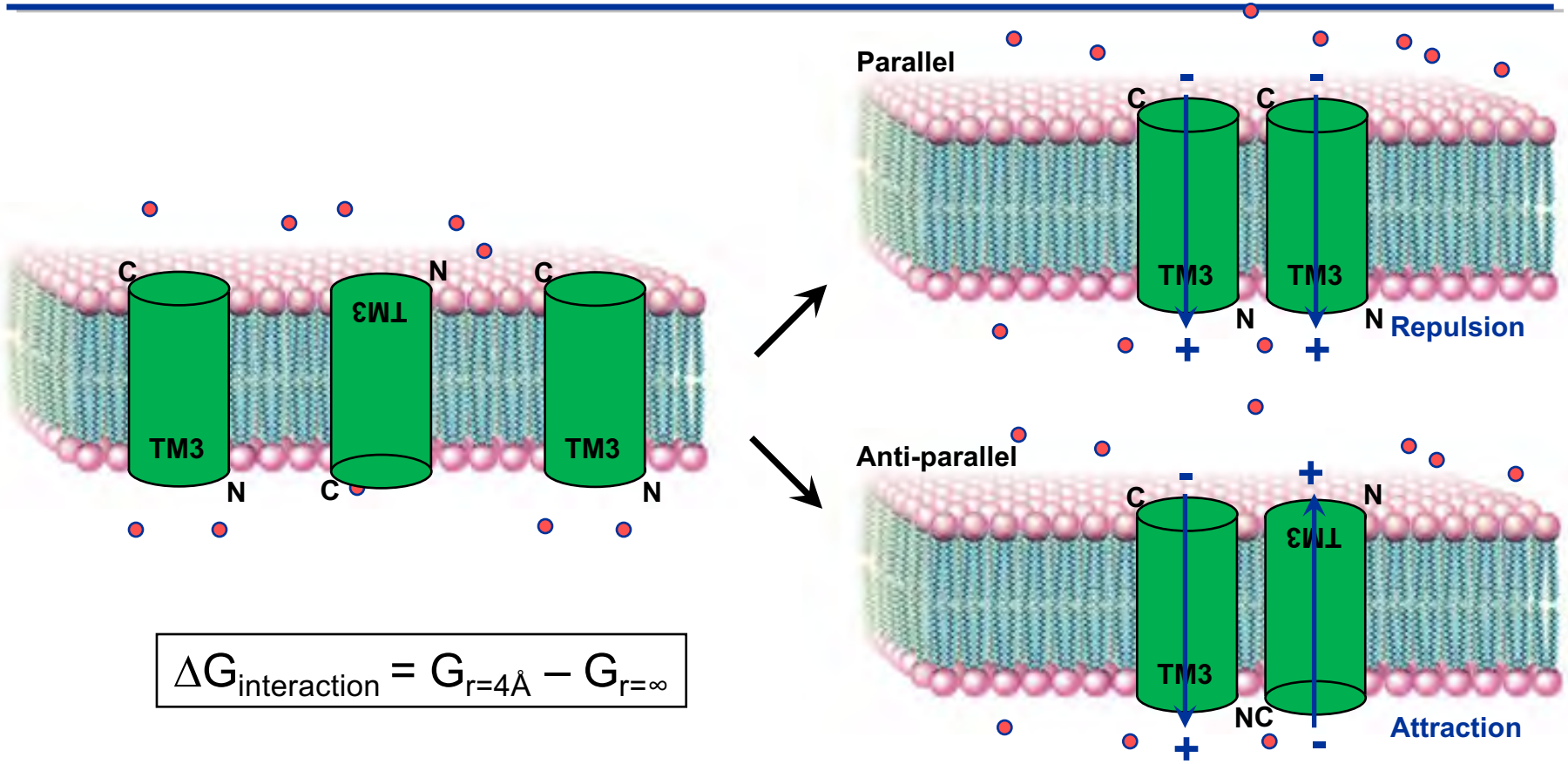
Electrostatics are also strong in lipid bilayers



$$\Delta G_{interaction} = G_{r=4Å} - G_{r=\infty}$$

Consider two transmembrane (TM) α-helices. TM1 has a Lys while TM2 has an Asp – both exposed to the hydrophobic lipid environment. There is no solvating water, so the two charges are not stable at r=∞ (*left*). In this case, the salt bridge will drive dimerization (right) because the charges are much more stable in a salt bridge than being "solvated" by the lipid bilayer. In this case $\Delta G_{int} <<< 0$.

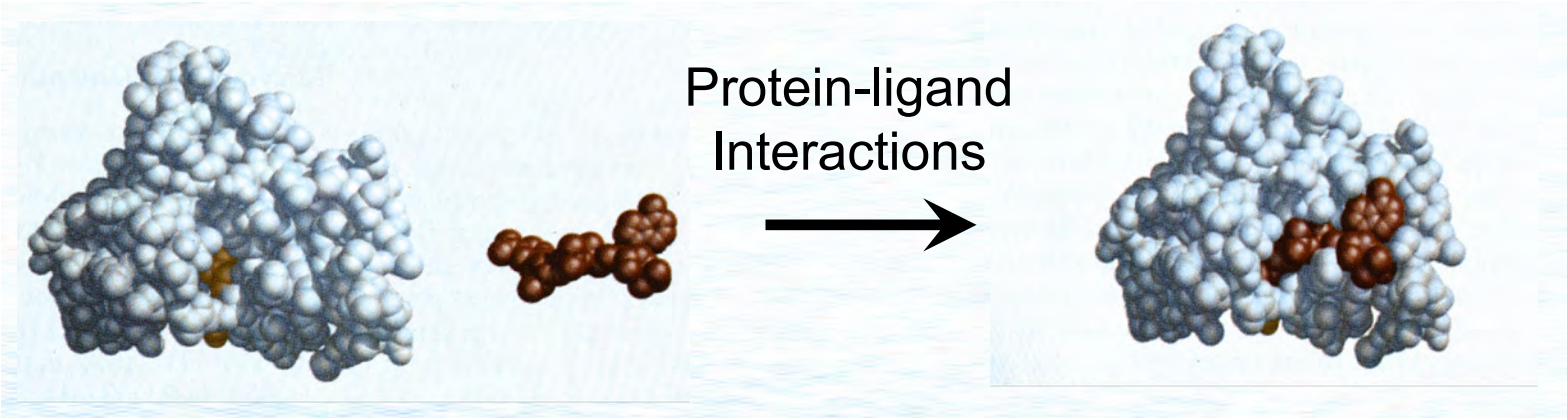
Electrostatics in a non-polar environment



$$\Delta G_{interaction} = G_{r=4Å} - G_{r=\infty}$$

Consider a bilayer containing TM α-helices. *All other interactions being equal*, dimerization would be dominated by dipole-dipole interactions, which favor the antiparallel orientation. In aqueous environments, dipole-dipole interactions are weak. In a bilayer they can be strong! *The strengths of all electrostatic interactions increase in a non-polar environment*

Conclusions:



We must always take into account solvent polarity when considering molecular interactions. In the case of protein folding or protein-ligand interactions (above), the solvent is aqueous and solvation/de-solvation plays a dominant role. In a lipid bilayer, the low dielectric constant magnifies the strengths of weak electrostatic interactions so that they have a tremendous influence. We will see that proteins have evolved sophisticated mechanisms to deal with the effects of solvent polarity and thus to maximize interaction energies for optimal function.